JOURNAL OF AGRICULTURAL RESEARCH

VOL. 46 WASHINGTON, D. C., APRIL 1, 1933 NO. 7

# HIBERNATION OF THE MEXICAN BEAN BEETLE IN THE ESTANCIA VALLEY, N. MEX.[1]

By J. R. DOUGLASS[2]

*Associate Entomologist, Division of Truck Crop and Garden Insects, Bureau of Entomology, United States Department of Agriculture*

## INTRODUCTION

The investigation described in this paper formed part of a comprehensive study of the hibernation of the Mexican bean beetle (*Epilachna corrupta* Muls.) made in the Estancia Valley, N. Mex., from 1923 to 1929. The investigation was undertaken to determine, if possible, the relative effects of hibernation material, snowfall coverage, temperature, precipitation, and climatic fluctuation on the overwintering of the beetle. In addition, information was sought which would be useful in determining the possible economic importance of this insect in noninfested bean-growing areas in advance of its spread to these areas.

Repeated observations have shown that the Mexican bean beetle is a major pest of beans only when they are grown near suitable hibernation quarters. In the Estancia Valley beetles begin their fall migration in search of hibernation quarters early in September and reach their maximum flight during the latter part of September or the first part of October, depending on the seasonal variation in weather conditions, especially the occurrence of killing frost, and on the time the bean plants are harvested. The beetles begin to leave their winter quarters and appear in the fields early in June. The peak of the infestation of overwintered beetles occurs in the foothill fields during July, the exact time depending on the quantity of rainfall. In this paper winter survival and overwintering refer to the entire hibernation period.

## METHODS OF INVESTIGATION

In these studies 216,340 beetles were used. They were collected from the following places: In 1923–24, from irrigated gardens in the Estancia Valley; in 1924–25, from the foothills of the Estancia Valley and the Rio Grande Valley, between Belen and Los Lunas, N. Mex.; in 1925–26 and 1926–27, from irrigated bean fields near Hoehne, Colo., and in the Rio Grande Valley north of Albuquerque, N. Mex.;

[1] Received for publication May 21, 1932; issued May, 1933.

[2] The writer's thanks are due J. E. Graf, formerly in charge of the Division of Truck-Crop Insects, Bureau of Entomology; W. H. White, in charge of the Division of Truck Crop and Garden Insects, and Neale F. Howard, in charge of the bean-insect investigations, for encouragement, inspiration, and suggestions; and to the temporary field assistants who were stationed at Estancia during the period of study discussed in the paper.

in 1927–28 and 1928–29, from the Rio Grande Valley, near Albuquerque and Belen.

In making the collections the plants were jarred with a thin board and the beetles fell into a specially constructed pan. After several hundred beetles had been collected, they were emptied into cloth sacks which the collectors carried in their belts. At intervals the contents of the sacks were dumped into a cage, in which they were transported to the laboratory by autotruck. At the laboratory the beetles were fed twice daily, the old refuse being removed from the cage at the time of feeding. Wet gunny sacks were placed over the cages to reduce the temperature and increase the humidity. The beetles were counted in the order of collection, so that each group was held at the laboratory approximately the same length of time. They were first placed in wide-mouth bottles and chilled in ice water to reduce their activity and permit rapid and accurate counting. When the desired number of beetles had been counted, they were placed in cylindrical carrying cases containing bean foliage and transferred to the hibernation cages in the field at a time when field beetles were seeking hibernation quarters.

During the season of emergence from hibernation, the cages in the foothills and valleys were observed as often as time and conditions would allow, generally daily or at least every other day. The cages located on the mountain were observed less frequently. After emergence had become general, the active beetles were removed from the cages on days when examinations were made and the numbers thus removed were recorded as the ones that had survived. These numbers were used in computing the percentages given in Table 2.

## DESCRIPTION OF CAGES

The hibernation cages were constructed of 2 by 4 inch lumber, and measured 4 feet wide, 6 feet long, and 2½ to 4 feet high. They were covered with 14-mesh screen wire and had removable tops. (Fig. 1.) After the beetles had become dormant, the tops were removed and the cages covered with 1-inch mesh wire. This permitted snow to enter the cages but excluded intruders. The original tops were replaced in the spring before the beetles resumed their activity. In this manner conditions closely approximating those prevailing in the surrounding area were secured. Extra heavy wire screening was placed vertically in the soil to a depth of 12 inches around the base of each cage to prevent burrowing animals from gaining access to the hibernating beetles. When the cages were placed in the soil, care was taken to preserve the forest floor or natural cover.

## LOCATION OF CAGES

Details as to the location of the hibernation cages are given in Table 1. Cage 1 was located near the upper edge of the fir-spruce association, or Canadian Zone, and cages 2 and 3 near the lower border of this zone. Cage 4 was placed at the upper edge of the ponderosa (western yellow) pine forest, or Transition, zone, and cages 5, 6, 7, and 8 in the lower half of this zone. Cage 9 was located in the piñon or nut-pine association that clothes the lower rolling foothills bordering the valley on the west and the ponderosa pine forest zone. Cages 10, 11, and 12 were placed in the short-grass or semidesert formation

of the Estancia Valley. The line of hibernation cages extended in an eastern and western direction for 25 miles and ascended from an elevation of 6,100 to one of 9,000 feet.

Cage 2 was placed against the base of a high rim rock, which afforded some protection and decreased the amount of precipitation entering the cage. Cages 5 and 7 were located on the south side of Tajique Canyon just below the first rim rock and were also well protected.

FIGURE 1.—Type of cage used in hibernation investigation, with instrument shelter in background

Cage 8 was placed at the base of a steep hill, 30 feet from the canyon stream, where it received a large amount of moisture from both precipitation and seepage. Cage 11 was set between the laboratory and the insectary, where it received the maximum quantity of snow and, being protected from the wind, the hibernation material was evenly covered. The remaining cages were not protected from the wind and in them the snow coverage was not evenly distributed over the hibernation material.

## WEATHER RECORDS

The temperature, precipitation, and snowfall records have been compiled from the meteorological records of cooperative observers of the United States Weather Bureau and from records taken at the Estancia laboratory. The three cooperating weather stations are located as follows: (1) At Rea's ranch, on Bosque Mountain, at an elevation of 9,215 feet, latitude 34° 46′ N., longitude 106° 20″ W., in the Canadian Zone, near cages 1 to 4, inclusive; (2) near Tajique, in the foothills at an elevation of 7,100 feet, latitude 34° 48′ N., longitude 106° 18″ W., 3 miles northeast of the foothill cages in the Transition Zone; (3) at Estancia, in the Upper Sonoran Zone, at an elevation of 6,100 feet, latitude 34° 45′ 20″ N., longitude 106° 3″ W., in the short-grass or semidesert formation, near cages 10, 11, and 12.

TABLE 1.—*Location of hibernation cages*

| Cage No. | Situation | Slope of situation | Elevation | Exposure | Topography | Soil | Environment | Plant association |
|---|---|---|---|---|---|---|---|---|
| | | | | CANADIAN ZONE | | | | |
| | | *Degrees* | *Feet* | | | | | |
| 1 | Mountain | 45 | 9,000 | North | Mountain side | Black loam | Dense forest of fir and spruce, a few maple and black locust. | Fir-spruce. |
| 2 | do | 45 | 8,400 | do | Midway on slope of mountain canyon. | do | Dense forest of scrub oak, maple, and a few fir and spruce. | Do. |
| 3 | do | 45 | 8,400 | do | do | do | do | Do. |
| | | | | TRANSITION ZONE | | | | |
| 4 | Mountain | 10 | 8,400 | Southeast | Mountain side | Black loam | Dense forest of scrub oak, a few pine, cedar, and black locust. | Pine. |
| 5 | Foothill | 20 | 7,050 | North | On side of broad canyon | do | Open forest of pine and white oak, dense near cage. | Do. |
| 6 | do | 20 | 7,050 | East | Hill, near center of slope | Sandy black loam | Open forest of pine and white oak, large pines near cage. | Do. |
| 7 | do | 7.5 | 7,000 | do | On side of broad canyon | Black loam | Open forest of pine and white oak | Do. |
| 8 | do | 45 | 6,975 | North | Near bottom of narrow canyon. | do | Open forest of pine and white oak, dense near cage. | Do. |
| | | | | UPPER SONORAN ZONE | | | | |
| 9 | Foothill | 2 | 6,800 | North | Hill, near top | Red clay loam | Open forest of cedar and piñon | Piñon-juniper. |
| 10 | Valley | 0 | 6,100 | Open | Broad valley | Sandy gray loam | Sod | Short grass or semidesert. |
| 11 | do | 0 | 6,100 | do | do | Black loam | Transplanting bed near insectary and laboratory | Do. |
| 12 | do | 0 | 6,100 | do | do | Sandy gray loam | Sod | Do. |

## GENERAL FEATURES OF ESTANCIA VALLEY

### PHYSIOGRAPHY

Estancia Valley [3] (fig. 2) extends from about latitude 34° 20′ to 35° 17′ N., and longitude 105° 42′ to 106° 24′ 30″ W. Its drainage basin forms a depression with no outlet, having a maximum extent of about 65 miles north and south and 40 miles east and west, and includes an area of about 2,000 square miles.

Four physiographic areas are recognized in the valley—the valley floor, hills, mesas, and mountains. Estancia Valley is separated from the Rio Grande Valley by the Manzano Mountain range, which extends north and south and parallels the two valleys for 30 miles as an unbroken mountain wall. This range has an abrupt slope fronting

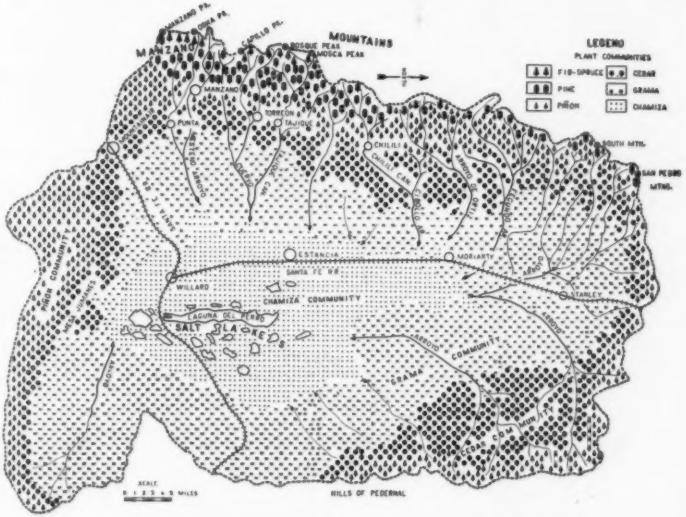


FIGURE 2.—Sketch of Estancia Valley, showing plant communities

the Rio Grande Valley by reason of an uplift which exposes the rock strata on its western slope in the form of a precipitous escarpment of about 4,000 feet. The long eastern slope follows the dip of these strata and gradually descends into the foothills of Estancia Valley. (Fig. 3.) At the northwest corner of the valley are South Mountain and the San Pedro Mountains, two isolated masses. Between South Mountain and the north end of the Manzano Range, a distance of nearly 15 miles, the mountain wall is interrupted, the divide between the Estancia and Rio Grande Valleys here being formed by a rugged hilly tract. From the center of the valley northward the surface rises gently to a point where the plain ends abruptly in an escarpment. On the northeast the valley is bordered by a mesa. Farther south are the Hills of Pedernal, which divide the valley from the treeless grass-

[3] MEINZER, O. E. GEOLOGY AND WATER RESOURCES OF ESTANCIA VALLEY, NEW MEXICO, WITH NOTES ON GROUND-WATER CONDITIONS IN ADJACENT PARTS OF CENTRAL NEW MEXICO. U. S. Geol. Survey Water-Supply Paper 275, 89 p., illus. 1911.

land plains of the Pecos slope. The hills that inclose the valley on the southwest are lower and less rugged. On the southeast the valley is terminated abruptly by the Mesa Jumanes, whose escarpment, 500 feet high, forms an imposing physiographic feature. The area drains into a series of large salt lakes in the southeastern part of the valley.

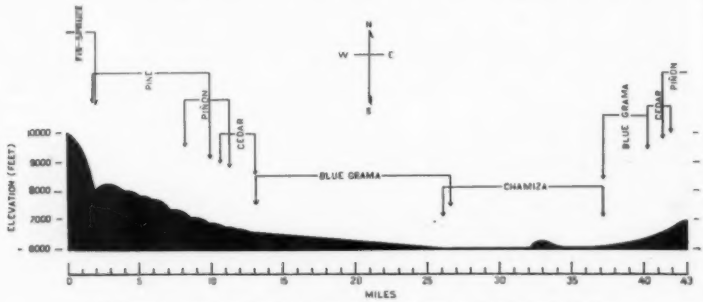


FIGURE 3.—Cross section of the plant associations in the Estancia Valley

The lowest point in the valley has an elevation of about 6,000 feet and the highest point in the Manzano Mountains is about 10,000 feet.

The climate of this area, with its cool summers and cold winters, is determined by its elevation, its inclosed nature, and the altitudinal range of approximately 4,000 feet. The highest and lowest temperatures occur at the lowest elevations.

## PLANT ASSOCIATIONS

The valley floor is divided into two plant zones, the alluvial slopes or plains and the ancient lake-bed region. The alluvial slopes are covered with a short-grass sod in which blue grama (*Bouteloua gracilis*)

FIGURE 4.—Blue grama (*Bouteloua gracilis*) sod

(fig. 4) predominates. The lake-bed area is dominated by a small-leafed shrub, fourwing saltbush (*Atriplex canescens*), commonly called chamiza in New Mexico. (Fig. 5.) In the fourwing-saltbush com-

munity are extensive areas that have a shallow water table and a high alkali content. These areas are covered by bluestem (*Andropogon furcatus*). Although most of the central part of the valley is flat,

FIGURE 5.—Fourwing saltbush (*Atriplex canescens*) that is found on the extensive areas of the old lake and the salty mud flats

extensive depressions containing salty mud flats (salt basins) devoid of vegetation are found.

The western foothills are divided into four forest zones (fig. 2), whereas the other sides of the valley afford only two. The lower roll-

FIGURE 6.—Cherrystone juniper (*Juniperus monosperma*) that sparsely clothes the lower rolling hills

ing hills that border the valley on the east and west are sparsely clothed with cherrystone juniper (*Juniperus monosperma*). (Fig. 6.) The mesa and the intermediate forest or nut-pine region are covered

with piñon (*Pinus edulis*). (Fig. 7.) (These types of vegetation fall within the Upper Sonoran Life Zone of Merriam.) The ponderosa pine (*Pinus ponderosa*) covers the higher rolling hills (Transition Zone)

FIGURE 7.—Piñon (*Pinus edulis*) that covers the mesa and intermediate forest or nut-pine region

along the foot of the Manzano Mountains and the lower and drier slopes of the mountains. (Fig. 8.) In places there are relatively open stands of this pine with a clean floor, but more often there is an under-

FIGURE 8.—Ponderosa pine (*Pinus ponderosa*) that covers the higher rolling hills and the lower and drier slopes of the mountains

growth of trees and shrubs, the most common being the oak (*Quercus gambelii*). In the elevated areas of the Manzano Mountains (Canadian Zone) the dominant evergreen trees are Douglas fir (*Pseudotsuga mucronata*) and Engelmann spruce (*Picea engelmanni*). White oaks

(*Quercus utahensis* and *Q. novomexicana*), Rocky Mountain maple (*Acer glabrum*), and quaking aspen (*Populus tremuloides*) are the deciduous trees occurring in greatest abundance.

## HIBERNATION OF BEETLES IN THE VARIOUS LIFE ZONES

### CANADIAN ZONE

The results obtained in the Canadian Zone in a period of five years show that in four of the years no beetles survived and in the fifth year (1926–27) only 42, or 0.56 per cent, survived. (Table 2.) During the 5-year period a total of 38,500 beetles was used, and during 1926–27, 7,500 beetles were used; the percentage survival for the five years was therefore 0.11. The winter of 1926–27 was unusually warm, with an average daily departure from normal temperature of 2° F. The precipitation was nearly normal.

In order to determine whether the character of the hibernation material was an important factor in the survival of beetles in this zone, pine needles were added to the oak leaves in cages 2 and 3 in the fall of 1926 and in the following years. (Table 2.) In 1927–28, when the average daily temperatures was 0.9° F. above normal and the precipitation 0.21 inch below normal (Table 9), no beetles survived. In 1928–29 the departures from normal were −1.4° F. and +0.26 inch of precipitation, and again no beetles survived. It is evident that in this zone winter mortality is due to climatic factors, and not to the character of the material used as a shelter for the beetles.

### TRANSITION ZONE

In the Transition Zone 133,340 beetles were used in six years, with a total survival of 13,114, or 9.84 per cent. At the upper edge of the ponderosa pine belt (cage 4) 5.01 per cent survived over a period of six years. In cages 5 and 7 more than 12 per cent survived the dormant period. The locations of these cages are typical of favorable natural hibernation quarters. Cage 8 showed the lowest percentage of survival in this zone, 3.16 per cent. It is doubtful whether beetles in search of hibernation quarters would under natural conditions attempt to hibernate in locations in which moisture conditions were so unfavorable as they were in this cage.

The results of the six years' experiments indicate that the ponderosa pine forest zone (fig. 8) is the natural hibernation quarters of the beetle in the West and that it is even more favorable when oak trees are present in the association. This indication is confirmed by the fact that beetles are found hibernating naturally chiefly in this zone.

### UPPER SONORAN ZONE

In the Upper Sonoran Zone 44,500 beetles were employed with a total survival of 1,210, or 2.72 per cent, in all four cages (Nos. 9 to 12) where materials both native and foreign to the zone were used as shelter. In cases 9 and 10, where the sheltering material was native to the zone, the percentage of beetles which hibernated successfully averaged 0.34. This average does not include the results for cage 9 in the season 1926–27, when the compact piñon needles in that cage were loosened in order to test the suitability of masses of needles as hibernation material. In the loosened needles 16.68 per cent of the insects

TABLE 2.—*Hibernation results, 1923–24 to 1928–29*

CANADIAN ZONE

| Cage No. | Season | Hibernation material | Date beetles were introduced | Beetles introduced | Beetles survived | |
|---|---|---|---|---|---|---|
| | | | | *Number* | *Number* | *Per cent* |
| 1 | 1924–25 | Spruce needles and cones | Sept. 30 | 5,000 | 0 | 0 |
| | 1925–26 | Maple leaves and spruce needles | Oct. 10 | 2,500 | 0 | 0 |
| | 1926–27 | do | Sept. 29 | 2,500 | 2 | .08 |
| | 1927–28 | do | Oct. 4 | 1,000 | 0 | 0 |
| | 1928–29 | do | Sept. 28 | 2,500 | 0 | 0 |
| 2 | 1924–25 | Oak leaves and fir needles | Oct. 2 | 3,000 | 0 | 0 |
| | 1925–26 | do | Oct. 8, 15 | 5,000 | 0 | 0 |
| | 1926–27 | Oak leaves and pine needles | Sept. 29 | 2,500 | 28 | 1.12 |
| | 1927–28 | do | Oct. 4 | 1,000 | 0 | 0 |
| | 1928–29 | do | Sept. 28 | 2,500 | 0 | 0 |
| 3 | 1925–26 | Oak leaves and fir needles | Oct. 8, 15 | 5,000 | 0 | 0 |
| | 1926–27 | Oak leaves and pine needles | Sept. 29 | 2,500 | 12 | .48 |
| | 1927–28 | do | Oct. 4 | 1,000 | 0 | 0 |
| | 1928–29 | do | Sept. 28 | 2,500 | 0 | 0 |

TRANSITION ZONE

| | | | | | | |
|---|---|---|---|---|---|---|
| 4 | 1923–24 | Oak leaves and pine needles | Oct. 9 | 5,004 | 208 | 4.16 |
| | 1924–25 | Oak leaves and cedar needles | Oct. 7 | 2,000 | 9 | .45 |
| | 1925–26 | do | Oct. 15 | 1,500 | 36 | 2.40 |
| | 1926–27 | do | Sept. 29 | 1,500 | 248 | 16.53 |
| | 1927–28 | do | Oct. 4 | 1,000 | 3 | .30 |
| | 1928–29 | do | Sept. 28 | 2,500 | 172 | 6.88 |
| 5 | 1924–25 | Oak leaves and pine needles | Oct. 15 | 1,296 | 130 | 10.03 |
| | 1925–26 | do | Oct. 10, 12 | 5,000 | 1,112 | 22.24 |
| | 1926–27 | do | Oct. 2 | 10,000 | 2,829 | 28.29 |
| | 1927–28 | do | Oct. 3, 5 | 10,000 | 75 | .75 |
| | 1928–29 | do | Sept. 29 | 10,000 | 295 | 2.95 |
| 6 | 1925–26 | Pine needles | Oct. 19 | 1,500 | 339 | 22.60 |
| | 1926–27 | do | Oct. 4 | 1,500 | [a] 206 | 13.73 |
| | 1927–28 | do | Oct. 6 | 2,500 | [b] 123 | 4.92 |
| | 1928–29 | do | Oct. 4 | 2,500 | [b] 38 | 1.52 |
| 7 | 1923–24 | Oak leaves and pine needles | Oct. 17 | 5,540 | 1,304 | 23.54 |
| | 1924–25 | do | Oct. 5, 7 | 5,000 | 363 | 7.26 |
| | 1925–26 | do | Oct. 11 | 5,000 | 1,812 | 36.24 |
| | 1926–27 | do | Oct. 3, 9 | 15,000 | 2,433 | 16.22 |
| | 1927–28 | do | Oct. 3, 5 | 10,000 | 230 | 2.30 |
| | 1928–29 | do | Oct. 4 | 10,000 | 358 | 3.58 |
| 8 | 1925–26 | do | Oct. 10 to 20 | 10,000 | 183 | 1.83 |
| | 1926–27 | do | Oct. 4 | 5,000 | 516 | 10.32 |
| | 1927–28 | do | Oct. 5 | 5,000 | 90 | 1.80 |
| | 1928–29 | do | Oct. 4 | 5,000 | 2 | .04 |

UPPER SONORAN ZONE

| | | | | | | |
|---|---|---|---|---|---|---|
| 9 | 1925–26 | Piñon needles | Oct. 11 | 2,500 | 70 | 2.80 |
| | 1926–27 | Piñon needles (loosened material) | Oct. 4 | 2,500 | 417 | 16.68 |
| | 1927–28 | Piñon needles | Oct. 6 | 2,500 | 0 | 0 |
| | 1928–29 | do | Oct. 4 | 2,500 | 0 | 0 |
| 10 | 1923–24 | Russian thistles and other weeds | Oct. 23 | 2,500 | 4 | .16 |
| | 1924–25 | Bean hulls and Russian thistles | Oct. 11 | 2,000 | 0 | 0 |
| | 1925–26 | do | Oct. 10 | 2,500 | 0 | 0 |
| | 1926–27 | do | Oct. 7 | 2,500 | 0 | 0 |
| | 1927–28 | do | do | 2,500 | 0 | 0 |
| | 1928–29 | do | Oct. 10 | 2,500 | 0 | 0 |
| 11 | 1925–26 | Oak leaves and pine needles | Oct. 18 | 2,500 | 545 | 21.80 |
| | 1926–27 | do | Oct. 7 | 5,000 | 103 | 2.06 |
| | 1927–28 | do | do | 2,500 | 23 | .92 |
| | 1928–29 | do | Oct. 4 | 2,500 | 28 | 1.12 |
| 12 | 1926–27 | do | Oct. 8 | 2,500 | 20 | .80 |
| | 1927–28 | do | Oct. 7 | 2,500 | 0 | 0 |
| | 1928–29 | do | Oct. 4 | 2,500 | 0 | 0 |

[a] On May 3 shelter placed over cage to exclude natural precipitation.
[b] On May 10 shelter placed over cage to exclude natural precipitation.

passed the winter successfully. In the three seasons that cage 9 contained compact masses of needles, not a single beetle survived during two seasons and only 2.8 per cent during another season, the average survival for the three seasons being 0.93 per cent. These results indicate that the natural hibernating material in the cherrystone juniper and piñon forest zone does not provide proper conditions for winter survival of the beetle, owing primarily to the fact that few of the beetles are able to enter the compact masses of needles. This conclusion conforms with observations made by Graf,[4] who did not find a single beetle hibernating in the piñon pine and cherrystone juniper needles in the foothills during the season of 1921–22, when the heaviest infestation of beetles ever known in the Estancia Valley was recorded. The needles of the piñon and cherrystone juniper trees are very short, and only a light fall occurs yearly, resulting in a scant compact mass around the base of each tree. Judging from the results obtained in cage 9 during 1926–27, however, it may be concluded that if the beetles were able to penetrate these masses of needles they would find proper protection for winter survival.

FIGURE 9.—Oak "mottes" found along the streams in the cedar and nut-pine forest zones

The results from cage 10, which contained materials common to the cultivated and sod areas of the Sonoran Zone, showed that there was only one season out of the six in which any beetles survived. Of the 14,500 beetles used in this experiment, 4 lived through the winter of 1923–24. Where oak leaves and pine needles, material which is foreign to the zone, were used as hibernation shelter (cages 11 and 12), 3.6 per cent of the beetles survived. Where protection from the wind was afforded, which allowed for an even distribution of snow and decreased sand coverage, as in cage 11, the percentage of beetles that overwintered averaged 5.59. In cage 12, which contained the same material but which was exposed to the wind, only 0.27 per cent passed the winter during the three years the experiment was in progress.

These facts show that sheltering material found naturally in the open areas of this zone does not provide the beetle with proper coverage for successful winter survival. The greater survival in the cages containing oak leaves and pine needles explains why beetles can hibernate in oak "mottes" (fig. 9) found along streams and

[4] Graf, J. E. Unpublished data.

canyons. The oak mottes represent an outcropping of the Transition Zone at the upper edge of the Upper Sonoran Zone due to topography, exposure, temperature, and soil moisture in that particular vicinity.

## MISCELLANEOUS FACTORS AFFECTING MORTALITY OF BEETLES

### DUST STORMS

In the Estancia Valley dust storms occur frequently in the spring and occasionally in other seasons. These storms may last for a few hours or for several days, and the quantity of dust carried at times is enormous. It has been found that the quantity of dust deposited is in direct proportion to the quantity and size of any obstruction in its path and the efficiency of this obstruction as a windbreak. Russian thistle (*Salsola pestifer*) is the most abundant and widely distributed weed in the Estancia Valley. When mature it breaks off at the surface of the ground and rolls before the wind. These weeds finally lodge and pile up along fences (fig. 10), where they

FIGURE 10.—Russian thistles (*Salsola pestifer*) piled up along a fence by the wind

break the force of the wind so that it deposits a large quantity of sand and dust on the leeward side of the fence, until the fence is finally covered. This accumulation alone prevents the successful hibernation of the bean beetle on the alluvial slopes and valley floor.

### CHARACTER OF HIBERNATION MATERIAL

The character of the hibernation material is an important factor in the successful overwintering of the insect. A suitable material is one that permits beetles to enter and emerge readily, and at the same time protects them both from low temperature and from rapid changes of temperature. It must also be able to retain moisture so that too rapid desiccation of the beetles will not occur. A mixture of oak leaves and pine needles, such as is often found in well-protected places, is the most favorable material. This finding confirms the

results of Howard and English [5] and Thomas [6] obtained in studies of the beetle in the Southern States. Compact material, such as juniper and piñon needles, is unsuitable because the beetles are unable to enter it. Weeds are the most unsatisfactory material because they permit rapid changes in temperature and moisture.

### SNOWFALL

A protection of snow during periods of subzero weather is essential for successful hibernation. The following example will illustrate this point. On December 19, 1927, a thermograph measuring air temperature registered −20° F., as compared with 21° (fig. 12, D) registered by a distance thermograph that was recording the temperature of the hibernation material of oak leaves and pine needles under 8 inches of snow in cage 7 near by. Again on February 10, 1929, the air temperature was −27° at Estancia, as compared with a temperature of 25° under a 6-inch blanket of snow in the hibernation material of oak leaves and pine needles in cage 11 near by. (Fig. 12, A.) At the same time the temperature in the hibernation material in the adjacent cage 10, where Russian thistles were used, was −1°. (Fig. 12, B.) Weeds hold the blanket of snow off the ground and permit lowering of temperature. The water content of 1 inch of snow may vary from 0.03 to over 0.10 inch,[7] according to temperature, altitude, etc., and this variation may affect its insulating properties.

### DRAINAGE

A comparison of the results obtained from cages 5 and 8 shows that drainage is a factor of importance in the successful hibernation of the beetle. Both cages were located on the northern exposure under similar climatic conditions and with the same type of hibernation material. The former cage had good drainage, while the latter stood on ground that was constantly wet by seepage. During the seasons of 1925–26 and 1926–27 the percentage survival in cage 5 was 22.24 and 28.29, respectively, while in cage 8 it was only 1.83 and 10.32.

### TIME OF KILLING FROST

The time at which the first killing frost occurs is an important factor in winter survival. From 1923 to 1928 the time of the first killing frost ranged from September 14 to October 22, with September 23 as the average. The growing season in the Estancia Valley is comparatively short, and the reproduction period of the Mexican bean beetle is still shorter, for the beetles do not emerge from hibernation until stimulated by the summer rains. Counts of overwintered beetles in the same field in the foothills for the six summers showed that the largest population of old beetles was present from July 8 to 20, with July 14 as the average date. The developmental period from egg to adult ranges from 40 to 45 days during the summer. When the killing frost occurs early and destroys the bean, which is the only host plant in the valley, a large percentage of the newly

[5] HOWARD, N. F., and ENGLISH, L. L. STUDIES OF THE MEXICAN BEAN BEETLE IN THE SOUTHEAST. U. S. Dept. Agr. Bul. 1243, 51 p., illus. 1924.
[6] THOMAS, F. L. LIFE HISTORY AND CONTROL OF THE MEXICAN BEAN BEETLE. Ala. Agr. Expt. Sta. Bul. 221, 99 p., illus. 1924.
[7] Data from correspondence with C. F. Linner, U. S. Weather Bureau, Santa Fe, N. Mex.

emerged beetles are forced into hibernation in a weakened condition, and the hibernation period is lengthened. On the other hand, a late killing frost prolongs the feeding period and shortens the hibernation period. It is therefore evident that a variation of 38 days in the occurrence of the killing frost in autumn is indirectly a factor in the successful hibernation of the insect.

### PROXIMITY OF HIBERNATION QUARTERS AND SEASONAL RAINFALL

In the West the Mexican bean beetle is not a major pest in any bean-growing district which does not have suitable hibernation quarters comparatively close to the bean area (within 50 to 70 miles) and which does not have a seasonal rainfall of at least 5 inches rather evenly distributed over the hibernation period. Furthermore, this beetle has never been reported as a serious pest where a well-defined dry hibernation season occurs, especially when the dry season is coincident with high temperatures. In the dry-farmed area in eastern Colorado, for example, the beetle is not known to occur, and in Union County, N. Mex., it has never been a pest of economic importance.

## HIBERNATION IN RELATION TO ALTITUDE

Considered independently of all other factors, and within certain limits, there seems to be no close relation between altitude and successful hibernation of the bean beetle, as is shown by the results for cages 4 and 7, located in the ponderosa pine zone. (Table 3.) In cage 4 a small quantity of juniper needles was used with oak leaves instead of pine needles as was the case in cages 7 and 11.

TABLE 3.—*Effect of altitude on hibernation of Mexican bean beetles*

| Cage No. | Elevation | Beetles surviving | | | | | |
|---|---|---|---|---|---|---|---|
| | | 1923–24 | 1924–25 | 1925–26 | 1926–27 | 1927–28 | 1928–29 |
| | *Feet* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 4 | 8,400 | 4.15 | 0.45 | 2.40 | 16.53 | 0.30 | 6.88 |
| 7 | 7,000 | 23.50 | 7.26 | 36.24 | 16.22 | 2.30 | 3.58 |
| 11 | 6,100 | ---------- | ---------- | 21.80 | 2.06 | .92 | 1.12 |

The data for 1923–24, 1924–25, 1925–26, and 1927–28 show that a larger percentage of the insects survived at the lower elevation than at the higher. The figures for 1926–27 and 1928–29, on the other hand, show a greater survival at the higher elevation. In the case of cage 11, at a still lower elevation, the survival was smaller for these two seasons, but larger in 1925–26 and 1927–28 than in cage 4 at the highest elevation.

These conflicting data indicate that elevation alone has no real significance within the zone of successful hibernation. It is known that the altitude of the ponderosa pine zone ranges from 6,850 to 8,400 feet on the eastern exposure and from 8,000 to 9,000 feet on the western exposure, with a great variation on the northern and southern exposures in the Manzano Mountain Range. Pearson [8] has found that temperature and moisture, not elevation, are the limiting factors in the distribution of trees in the Southwest.

[8] PEARSON, G. A. FOREST TYPES IN THE SOUTHWEST AS DETERMINED BY CLIMATE AND SOIL. U. S. Dept. Agr. Tech. Bul. 247, 144 p., illus. 1931.

## RELATION OF EXPOSURE TO SURVIVAL OF BEETLES

The results of six seasons' work, as shown in Table 4, justify the conclusion that exposure, at least so far as locations on eastern, southeastern, and northern slopes are concerned, affects the survival of beetles under mountainous conditions in New Mexico. The important factor in this connection seems to be the topography of the locality as it governs the intensity or degree of shade. Where the hills are low and the slope is comparatively short, as is the case with the situation of cages 5 and 7, exposure considered apart from other factors seems to have no real significance within the zone of hibernation, but it has an important bearing upon beetle mortality in hibernation during certain seasons. The locations of cages 5 and 7 were similar as to topography, drainage, elevation, flora, soil, and hibernation material, and their close proximity permitted precipitation stimulus to affect the beetles in the two cages similarly.[9] The only important difference was in exposure.

TABLE 4.—*Effect of elevation and exposure on hibernation of Mexican bean beetles*

| Cage No. | Elevation | Exposure | Beetles surviving | | | | | |
|---|---|---|---|---|---|---|---|---|
| | | | 1923–24 | 1924–25 | 1925–26 | 1926–27 | 1927–28 | 1928–29 |
| | *Feet* | | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 2 | 8,400 | North | ---------- | 0 | 0 | 1.12 | 0 | 0 |
| 4 | 8,400 | Southeast | 4.15 | 0.45 | 2.40 | 16.53 | 0.30 | 6.88 |
| 5 | 7,050 | North | ---------- | 10.03 | 22.24 | 28.29 | .75 | 2.95 |
| 7 | 7,000 | East | 23.50 | 7.26 | 36.24 | 16.22 | 2.30 | 3.58 |
| 8 | 6,975 | North | ---------- | ---------- | 1.83 | 10.32 | 1.80 | .04 |

There was no cage in operation on the northern slope during the season of 1923–24. During the winters of 1925–26, 1927–28, and 1928–29 more insects survived in cage 7 on the eastern slope than in cage 5 on the northern. In 1924–25 and 1926–27, however, more beetles hibernated successfully on the northern slope. The average survival for the five winters shows no significant difference for the two exposures, being 12.24 per cent for the northern and 11.55 per cent for the eastern. When each year is considered separately, however, and such other factors as sunlight, evaporation, temperature, and soil moisture are taken into consideration, it is evident that exposure has a direct influence on the beetle during its dormant period. It will be shown later that in 1924–25 and 1926–27, when more beetles survived on the northern than on the eastern slope, the winters were followed by mild, dry springs, whereas in 1925–26, 1927–28, and 1928–29, when more beetles hibernated on the eastern slope, the springs were cold and wet. A study of beetle hibernation on any one exposure will therefore not give a true index of winter mortality from season to season, as is indicated in Figure 11.

Where the slope is steep and the distance from the cage to the top of the slope is comparatively great, as in cages 2 and 4, exposure considered apart from other factors seems to have a real significance. These two cages were located at the same elevation less than 300 yards

[9] DOUGLASS, J. R. PRECIPITATION AS A FACTOR IN THE EMERGENCE OF EPILACHNA CORRUPTA FROM HIBERNATION. Jour. Econ. Ent. 21: 203–213, illus. 1928.

apart on an eastern spur of Bosque Mountain. Cage 2 was near the lower edge of the fir-spruce association and cage 4 at the upper edge of the ponderosa pine zone, as in this vicinity the Canadian and Transition Zones dovetail into each other on account of the topography and slope exposure. The general air temperature and the precipitation occurring over the two cages were obviously very nearly the same. Cage 4, located on the southeastern slope, was exposed to the sun's rays longer than cage 2 on the northern slope; consequently, the evaporation was greater and the temperature of the duff higher on the southeastern slope. Because of the lower temperature the snow lasts longer on the northern slope, which makes it appear to have more

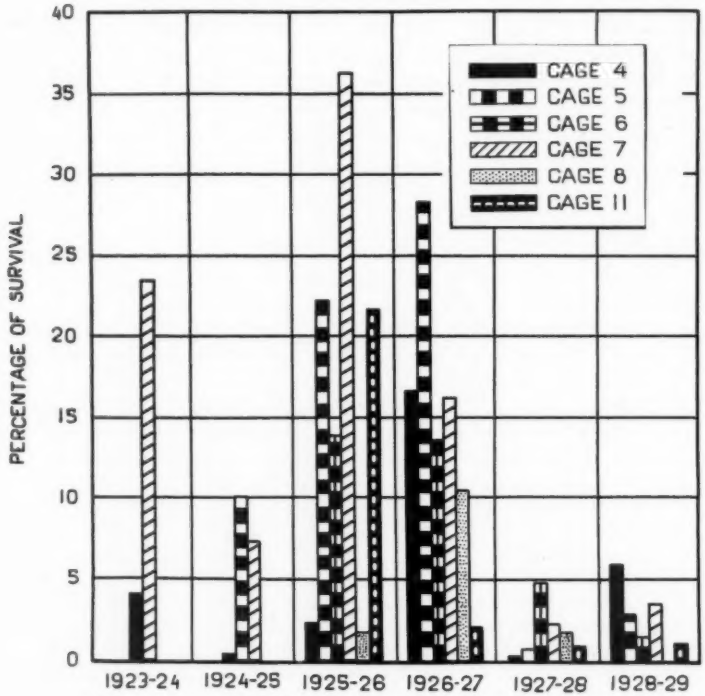


FIGURE 11.—Percentage of beetles surviving hibernation in different cages

moisture than the warmer slopes of the mountain. During the last five seasons of the experimental work 14,000 beetles were introduced into cage 2 and 8,500 into cage 4. In cage 2, 28 beetles survived and in cage 4, 468. (Table 2.) From these results it is apparent that at the same elevation in cages 300 yards apart more beetles may survive in one exposure than in the other.

The important part played by slope exposure and topography in winter mortality may be shown in still a different way by comparing the data given in Tables 1 and 2 for cage 8 with those for the neighboring cage 7. During the four seasons beginning with 1925–26,

the average survival of the 40,000 beetles in cage 7 on the eastern slope was 12.08 per cent as compared with 3.16 per cent for the 25,000 beetles in cage 8 on the northern slope. These data indicate that within the lower part of the ponderosa pine zone, the beetle's natural hibernation quarters in the West, there are certain localized areas that are not suitable for successful overwintering on account of the topography and slope exposure.

## HIBERNATION IN RELATION TO TEMPERATURE

A study of the monthly minimum temperatures at Estancia and Tajique (Table 5) for the hibernation seasons under discussion, in connection with Table 2, shows that the lowest temperatures occurred in years that were favorable to hibernation. During the winter of 1924–25, when −17° F. was recorded for Tajique, 10.03 per cent of the beetles introduced into cage 5 overwintered. Again during the season of 1925–26, −24° was recorded at Estancia and 21.8 per cent of the beetles survived in cage 11. The lowest temperature (−26°) occurred at Estancia and yet beetles survived this low air temperature, while in the fir-spruce association on Bosque Mountain (Rea's ranch), where the lowest temperature recorded in five years was −15°, there was 100 per cent mortality in the cages in four out of five years of the investigational period.

TABLE 5.—*Monthly minimum temperatures at Estancia and Tajique, N. Mex., and the lowest monthly temperatures recorded at Rea's ranch, 1923–24 to 1928–29*

| Station | Season | October | November | December | January | February | March | April | May | June |
|---|---|---|---|---|---|---|---|---|---|---|
| | | °F. | °F. | °F. | °F. | °F. | °F. | °F. | °F. | °F. |
| Estancia | 1923–24 | 23 | 1 | −16 | −2 | −2 | 8 | 15 | 27 | 33 |
| | 1924–25 | 17 | 2 | −26 | −11 | 8 | 3 | 21 | 27 | 30 |
| | 1925–26 | 11 | 4 | −5 | −24 | 5 | 0 | 20 | 23 | 39 |
| | 1926–27 | 18 | 12 | −16 | −1 | 12 | 10 | 19 | 28 | 31 |
| | 1927–28 | 17 | 4 | −10 | −5 | −5 | 11 | 1 | 29 | 35 |
| | 1928–29 | 21 | 13 | −1 | −4 | −22 | 8 | 15 | 17 | 36 |
| Tajique | 1923–24 | 12 | −2 | −2 | 2 | −1 | 3 | 13 | 24 | 31 |
| | 1924–25 | 20 | 10 | −17 | −4 | 14 | 10 | 22 | 32 | 33 |
| | 1925–26 | 24 | 4 | 0 | −4 | 10 | 0 | 24 | 23 | 38 |
| | 1926–27 | 15 | 15 | −10 | 12 | 13 | 5 | 20 | 26 | 36 |
| | 1927–28 | 20 | 13 | −13 | −4 | −3 | 8 | −4 | 30 | 34 |
| | 1928–29 | 19 | 10 | −1 | −2 | −13 | 1 | 13 | 13 | 34 |
| Estancia | Lowest | 11 | 1 | −26 | −24 | −22 | 0 | 1 | 17 | 30 |
| Tajique | do | 12 | −2 | −17 | −4 | −13 | 0 | −4 | 13 | 31 |
| Rea's ranch [a] | do | 14 | 0 | −6 | −8 | −15 | −1 | 10 | 12 | 23 |

[a] Temperatures for period 1913 to 1917; no weather station in operation during the investigational period.

The weekly thermograph records showing the temperatures of the air and of the hibernation material, as presented in Figure 12, illustrate why beetles under the cover of hibernation materials are able to survive during periods when the air temperatures above the materials are extremely low. During periods of subzero weather the ground is usually covered with snow, the protection of which is discussed elsewhere in this paper. The records for April 9, 1928, and May 2, 1929, show that the high mortality occurring during the seasons of 1927–28 and 1928–29 was not caused by low air temperature on these dates, as the temperature of the hibernation material did not go below 30° F.

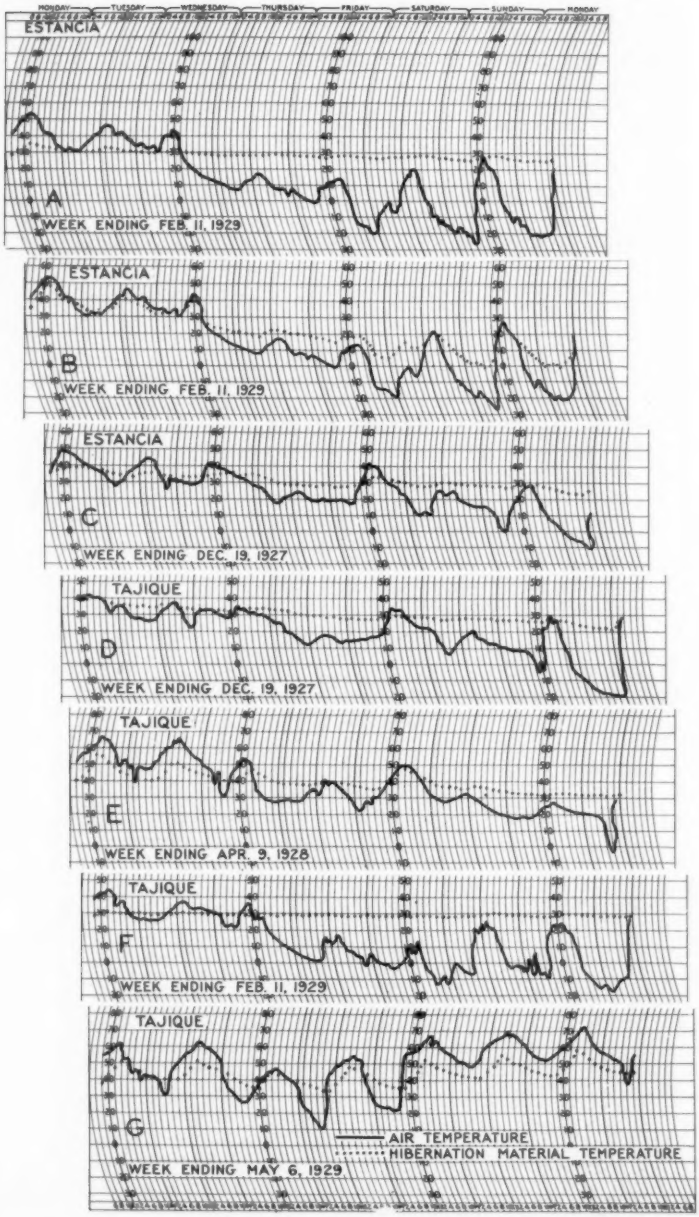


FIGURE 12.—Weekly thermograph records showing the temperatures of the air and of the hibernation material. A is a record taken in cage 11, in which the hibernation material consisted of oak leaves and pine needles, whereas B is a record for the same period in adjacent cage 10, where Russian thistles were used. In all other cases the hibernation material consisted of oak leaves and pine needles

In Table 6 and Figure 13 it is found that the mean minimum temperatures for November, December, and January are lower at Estancia than at Rea's ranch on Bosque Mountain or in the foothills (Tajique), while from February to June, inclusive, these temperatures are lower on the mountain than in the foothills or valley. Of the beetles introduced over a period of five years into cages located on the mountain (Nos. 1, 2, and 3), none survived except during the season of 1926–27, when the monthly mean minimum temperature from January to April, inclusive, at Estancia and Tajique rose 19.3° and 15.5° F., respectively, above the average mean for this period. This mild season, which prevailed generally over the area, permitted successful hibernation even at the higher elevations. This is best illustrated by the results obtained in cage 4 at 8,400 feet elevation, where the average survival for the mild season of 1926–27 was 16.53 per cent as compared with 3.57 per cent for the other five seasons. In cage 8 also during the mild season 10.32 per cent of the beetles survived as compared with an average of 1.38 per cent for the season preceding and the two seasons following the mild winter. It will therefore be seen that the temperatures of the latter part of the hibernating period have the greatest influence on survival.

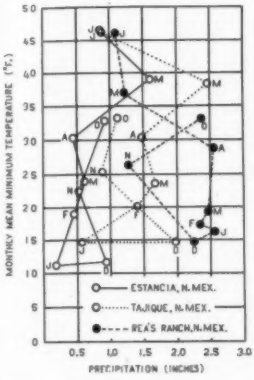


FIGURE 13.—Climograph showing mean monthly minimum temperatures at Estancia, Tajique, and Rea's ranch, N. Mex.

TABLE 6.—*Monthly mean minimum temperatures at Estancia and Tajique, N. Mex., and the mean of the monthly mean minimum temperatures there and at Rea's ranch, 1923–24 to 1928–29*

| Station | Season | October | November | December | January | February | March | April | May | June |
|---|---|---|---|---|---|---|---|---|---|---|
| | | °F. | °F. | °F. | °F. | °F. | °F. | °F. | °F. | °F. |
| Estancia | 1923–24 | 32.8 | 23.3 | 15.8 | 12.1 | 18.4 | 21.7 | 29.2 | 39.4 | 47.1 |
| | 1924–25 | 30.3 | 19.4 | 7.2 | 8.8 | 20.6 | 22.4 | 30.2 | 39.8 | 46.2 |
| | 1925–26 | 33.3 | 17.1 | 9.2 | −2.1 | 18.3 | 22.1 | 30.8 | 36.0 | 45.8 |
| | 1926–27 | 33.8 | 24.0 | 9.8 | 21.8 | 25.5 | 24.2 | 32.1 | 37.5 | 45.6 |
| | 1927–28 | 28.2 | 25.6 | 11.3 | 11.3 | 19.6 | 26.0 | 30.1 | 40.7 | 46.9 |
| | 1928–29 | 37.5 | 25.0 | 15.6 | 15.1 | 11.9 | 26.4 | 29.4 | 40.4 | 47.2 |
| Tajique | 1923–24 | 31.1 | 22.6 | 16.6 | 13.0 | 19.6 | 20.3 | 27.8 | 38.0 | 46.9 |
| | 1924–25 | 30.8 | 24.4 | 13.2 | 11.1 | 23.8 | 26.8 | 32.6 | 41.6 | 47.1 |
| | 1925–26 | 35.3 | 23.4 | 16.6 | 10.9 | 23.0 | 22.0 | 33.0 | 26.6 | 44.2 |
| | 1926–27 | 35.3 | 27.2 | 14.2 | 21.4 | 26.3 | 23.9 | 32.8 | 37.4 | 43.2 |
| | 1927–28 | 31.1 | 30.8 | 12.9 | 19.1 | 16.7 | 25.4 | 28.6 | 38.8 | 50.4 |
| | 1928–29 | 35.3 | 22.5 | 15.1 | 13.4 | 11.2 | 23.1 | 27.8 | 37.9 | 46.0 |
| Estancia | Mean | 32.7 | 22.4 | 11.5 | 11.2 | 19.0 | 24.0 | 30.3 | 39.0 | 46.5 |
| Tajique | do | 33.2 | 25.2 | 14.8 | 14.8 | 20.1 | 23.6 | 30.4 | 38.4 | 46.3 |
| Rea's ranch * | do | 33.0 | 26.4 | 14.8 | 16.3 | 17.3 | 19.3 | 28.9 | 37.0 | 46.9 |

* Data recorded between 1913 and 1917.

A further study of Table 6 shows that for Estancia during the hibernation season of 1925–26 the sum of the monthly mean minimum temperatures for November to March, inclusive, decreased 23.5° F. from the sum of the means for the same period. During this season

21.80 per cent of the beetles introduced into cage 11 survived as compared with an average survival of 1.54 per cent for the three succeeding years. (Table 2.) These conflicting data show that other important factors besides air temperature are exerting an influence upon the winter mortality of bean beetles.

## HIBERNATION IN RELATION TO TEMPERATURE AND PRECIPITATION

The moisture requirements of the beetles vary directly with the temperature, as is indicated in Table 7, which gives the relation between beetle survival and the average seasonal temperature and precipitation at stations in various parts of the range of the beetle in this country.

TABLE 7.—*Average seasonal temperature, precipitation, and beetle survival at several stations*

| Station | Period | Hibernation period | Temperature | Precipitation | Beetle survival |
|---|---|---|---|---|---|
| | | | °F. | *Inches* | *Per cent* |
| Tajique, N. Mex. | 1923 to 1929 | October to June | 40.35 | 12.47 | [a] 12.60 |
| Rea's ranch, N. Mex. | 1924 to 1929 | do | 35.94 | 18.08 | .11 |
| Columbus, Ohio | 1925 to 1929 | October to May | 42.71 | 23.76 | [b] 1.43 |
| Amesville, Ohio | do | do | 43.94 | [c] 24.64 | [b] 2.38 |
| Birmingham, Ala. | 1920 to 1928 | November to April | 52.83 | 30.36 | [b] 15.41 |
| Clemson College, S. C. | 1924 to 1929 | do | 50.12 | 27.43 | [d] 15.76 |

[a] For cages 5 and 7.
[b] From unpublished data by N. F. Howard, Bureau of Entomology.
[c] For Athens, Ohio.
[d] Calculated from data given by EDDY, C. O., and CLARKE, W. H. THE MEXICAN BEAN BEETLE, 1927–1928. S. C. Agr. Expt. Sta. Bul. 258, 42 p., illus. 1929.

The climographs for these stations, presented in Figure 14, show the average monthly temperature and precipitation during the hibernation period under discussion.

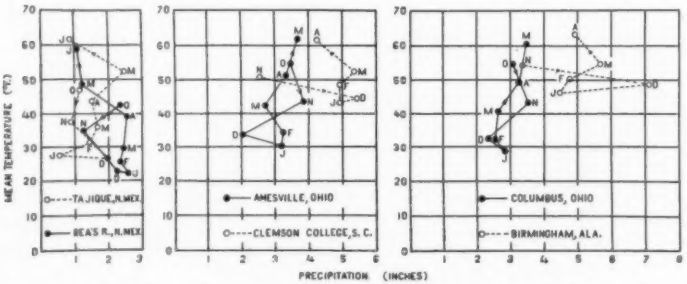


FIGURE 14.—Climograph showing mean monthly temperatures and precipitation in different parts of the range of the Mexican bean beetle in the United States

The average temperature at Tajique, N. Mex., is 2.36° F. lower than at Columbus, Ohio. From the standpoint of temperature, therefore, Columbus is more favorable for winter survival, but exces-

sive moisture combined with low temperature makes it unfavorable for successful hibernation. Tajique, on the other hand, with 11.29 inches less moisture, is favorable for survival. There is little difference in the percentage survival at Birmingham, Ala., and Tajique, N. Mex.; yet the latter station is 12.48° colder and has 17.89 inches less precipitation.

A few beetles have lived 3 days submerged in running water, although the mortality was high on the first and second days. Beetles in perforated salve boxes containing oak leaves placed under a dripping faucet lived from 1 to 12 days, but heavy mortality occurred between the first and fourth days.

## HIBERNATION AS AFFECTED BY CLIMATIC FLUCTUATIONS

### SEASONAL FLUCTUATIONS

The data in Table 8 and the climographs for Estancia and Tajique (fig. 15) show large variations, from season to season, in temperature and precipitation for corresponding months during the hibernation

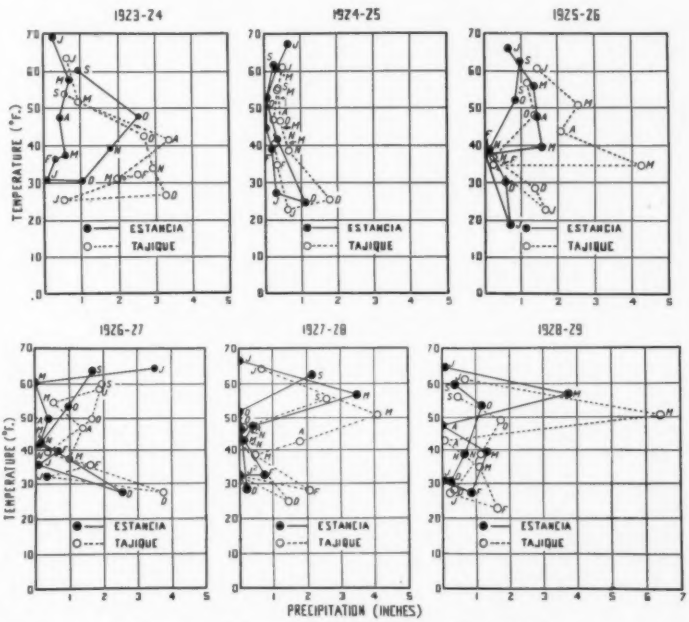


FIGURE 15.—Climographs for Estancia and Tajique, N. Mex.

period. This fluctuation in climatic conditions seems to be the most important factor affecting hibernation in the same cage. A single departure from the normal is not always important, but the combination or the sequence of such departures is important.

TABLE 8.—*Climatic data for Estancia and Tajique, N. Mex., 1923–24 to 1928–29*

MONTHLY MEAN TEMPERATURES

| Station | Hibernation season | Sept. | Oct | Nov. | Dec. | Jan. | Feb. | Mar. | Apr. | May | June | July |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | ° F. | ° F. | ° F. | ° F. | ° F. | ° F. | ° F. | ° F. | ° F. | ° F. | ° F. |
| Estancia | 1923–24 | 60.3 | 47.8 | 39.3 | 30.2 | 30.7 | 36.2 | 37.1 | 47.5 | 57.8 | 69.1 | 69.7 |
| | 1924–25 | 61.8 | 52.1 | 41.6 | 24.8 | 27.2 | 39.0 | 44.7 | 52.9 | 60.7 | 67.1 | 72.6 |
| | 1925–26 | 62.3 | 52.0 | 38.9 | 30.1 | 18.8 | 37.6 | 39.5 | 47.7 | 55.7 | 66.0 | 67.4 |
| | 1926–27 | 63.9 | 53.6 | 42.1 | 27.3 | 35.6 | 39.8 | 41.3 | 50.0 | 60.2 | 64.5 | 70.4 |
| | 1927–28 | 62.5 | 51.8 | 45.6 | 28.2 | 32.5 | 32.4 | 42.9 | 47.1 | 56.8 | 66.6 | 68.4 |
| | 1928–29 | 59.7 | 53.2 | 38.4 | 30.3 | 30.5 | 27.1 | 39.2 | 47.5 | 56.8 | 64.7 | 68.5 |
| Tajique | 1923–24 | 53.6 | 42.4 | 33.8 | 26.8 | 25.2 | 32.0 | 31.0 | 41.4 | 51.8 | 63.6 | 62.4 |
| | 1924–25 | 55.4 | 46.2 | 38.7 | 25.4 | 22.8 | 34.2 | 40.2 | 46.6 | 54.8 | 61.0 | 64.8 |
| | 1925–26 | 56.7 | 48.0 | 36.5 | 28.5 | 22.8 | 34.6 | 34.2 | 43.6 | 50.8 | 60.6 | 61.8 |
| | 1926–27 | 60.0 | 49.5 | 39.1 | 27.1 | 33.5 | 35.2 | 37.2 | 46.4 | 54.6 | 58.6 | 64.2 |
| | 1927–28 | 55.6 | 46.8 | 42.4 | 24.9 | 32.4 | 27.9 | 38.1 | 42.2 | 50.9 | 64.4 | 64.3 |
| | 1928–29 | 56.0 | 49.0 | 34.3 | 28.0 | 26.9 | 22.9 | 34.8 | 42.8 | 50.7 | 61.0 | 62.9 |

MONTHLY PRECIPITATION

| | | Inches | Inches | Inches | Inches | Inches | Inches | Inches | Inches | Inches | Inches | Inches |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Estancia | 1923–24 | 0.91 | 2.51 | 1.78 | 1.02 | 0.07 | 0.30 | 0.59 | 0.42 | 0.70 | 0.26 | 1.88 |
| | 1924–25 | .24 | .05 | .32 | 1.09 | .30 | .20 | .04 | .08 | .33 | .65 | 2.03 |
| | 1925–26 | .99 | .82 | .13 | .58 | .70 | .02 | 1.54 | 1.43 | 1.37 | .63 | 2.20 |
| | 1926–27 | 1.65 | .97 | .15 | 2.54 | .06 | .64 | .05 | .37 | (a) | 3.50 | 2.30 |
| | 1927–28 | 2.13 | (a) | .02 | .20 | 0 | .73 | .13 | .39 | 3.47 | (a) | .74 |
| | 1928–29 | .36 | 1.14 | .67 | .23 | .03 | .88 | 1.31 | (a) | 3.75 | .07 | 5.47 |
| Tajique | 1923–24 | .57 | 2.66 | 2.90 | 3.23 | .53 | 2.49 | 1.98 | 3.35 | .92 | .62 | 5.41 |
| | 1924–25 | .40 | .43 | .65 | 1.77 | .61 | .44 | .26 | .24 | .38 | .51 | 2.62 |
| | 1925–26 | 1.14 | 1.37 | .23 | 1.39 | 1.69 | .23 | 4.24 | 2.09 | 2.55 | 1.47 | 2.14 |
| | 1926–27 | 1.95 | 1.63 | .35 | 3.77 | .32 | 1.60 | .99 | 1.38 | .51 | 1.86 | 1.70 |
| | 1927–28 | 2.57 | .11 | 0 | 1.43 | 0 | 2.06 | .44 | 1.80 | 4.08 | .05 | 2.98 |
| | 1928–29 | .47 | 1.72 | 1.13 | .31 | .21 | 1.63 | 2.09 | .05 | 6.42 | .68 | 6.50 |

TOTAL MONTHLY SNOWFALL (UNMELTED)

| | | | Inches | Inches | Inches | Inches | Inches | Inches | Inches | Inches | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Estancia | 1923–24 | ..... | 4.5 | 4.5 | 13.5 | 1.0 | 3.9 | 10.2 | 2.2 | 0 | ..... | ..... |
| | 1924–25 | ..... | 0 | 0 | 13.2 | 7.2 | 1.5 | 0 | (a) | 0 | ..... | ..... |
| | 1925–26 | ..... | (a) | .5 | 5.0 | 10.0 | .2 | 10.5 | 0 | 0 | ..... | ..... |
| | 1926–27 | ..... | 2.0 | 0 | 9.3 | (a) | 1.0 | .2 | 1.0 | 0 | ..... | ..... |
| | 1927–28 | ..... | 0 | 0 | 4.8 | 0 | 12.0 | .5 | 4.5 | (a) | ..... | ..... |
| | 1928–29 | ..... | 0 | 3.0 | 1.0 | (a) | 18.0 | 10.0 | 0 | 1.0 | ..... | ..... |
| Tajique | 1923–24 | ..... | 25.5 | 21.0 | 46.5 | 7.0 | 29.0 | 19.7 | 20.7 | .5 | ..... | ..... |
| | 1924–25 | ..... | .5 | 0 | 27.2 | 11.3 | 3.3 | .5 | 0 | 0 | ..... | ..... |
| | 1925–26 | ..... | (a) | 3.0 | 13.5 | 24.6 | 2.7 | 37.8 | (a) | (a) | ..... | ..... |
| | 1926–27 | ..... | 5.5 | .8 | 39.3 | 3.0 | 8.1 | 8.0 | 13.0 | 1.0 | ..... | ..... |
| | 1927–28 | ..... | 0 | 0 | 21.2 | 0 | 31.2 | (a) | 17.1 | 19.4 | ..... | ..... |
| | 1928–29 | ..... | .2 | 9.5 | 4.8 | 3.9 | 26.6 | 19.0 | (a) | 2.0 | ..... | ..... |

[a] Trace.

The departures from the normal of temperature and precipitation at Tajique are shown in Table 9.

During the season of 1923–24 the fall was relatively cold and wet, and it was followed by a cold, damp winter with the heaviest snowfall on record. May was nearly normal in temperature, but far below normal in precipitation. June was hot and dry, so that emergence from hibernation was delayed until the July rains. As a whole the hibernation season was the coldest and wettest recorded. However, the winter was favorable for hibernation because the beetles were well protected by a heavy blanket of snow during the cold months, and a mild dry spring followed which was unfavorable for the parasitic fungus *Beauveria globulifera*. In cage 7, on an eastern slope, 23.5 per cent of the beetles survived. It is thus apparent that certain determining factors (in the present instance temperature and pre-

cipitation as they affect the development of parasitic fungi) may be so influenced by other determining factors, such as form of precipitation (snow instead of rainfall) or the time of occurrence of the precipitation, as to counteract the unfavorable effects of the first factors.

TABLE 9.—*Departures from the normal of temperature and precipitation, at Tajique, N. Mex., 1923–24 to 1928–29*

[7,100 feet elevation]

TEMPERATURE

| Month | 1923–24 | 1924–25 | 1925–26 | 1926–27 | 1927–28 | 1928–29 |
|---|---|---|---|---|---|---|
| | ° *F.* | ° *F.* | ° *F.* | ° *F.* | ° *F.* | ° *F.* |
| October | −4.2 | −0.4 | +1.4 | +2.9 | +0.2 | +2.4 |
| November | −3.3 | +1.6 | −.6 | +2.0 | +5.3 | −2.9 |
| December | −1.8 | −3.2 | −.1 | −1.5 | −3.7 | −.6 |
| January | −3.1 | −5.5 | −5.5 | +5.2 | +4.1 | −1.5 |
| February | +1.0 | +3.2 | +3.6 | +4.2 | −3.1 | −8.1 |
| March | −4.8 | +4.4 | −1.6 | +1.4 | +2.3 | −1.0 |
| April | −1.8 | +3.4 | +.4 | +3.2 | +1.0 | +.6 |
| May | −.4 | +2.6 | −1.4 | +2.4 | −1.3 | −1.5 |
| June | +2.7 | +.1 | −.3 | −2.3 | +3.5 | −.1 |
| Seasonal average | −1.8 | +.7 | −.5 | +2.0 | +.9 | −1.4 |

PRECIPITATION

| | *Inches* | *Inches* | *Inches* | *Inches* | *Inches* | *Inches* |
|---|---|---|---|---|---|---|
| October | +1.34 | −0.87 | +0.05 | +0.31 | −1.21 | +0.40 |
| November | +2.11 | −.14 | −.56 | −.44 | −.79 | +.34 |
| December | +1.75 | +.29 | −.09 | +2.29 | −.05 | −1.17 |
| January | −.39 | −.31 | +.77 | −.60 | −.92 | −.71 |
| February | +1.24 | −.81 | −1.02 | +.35 | +.81 | +.38 |
| March | +.55 | −1.17 | +2.81 | −.44 | −.99 | +.66 |
| April | +1.85 | −1.26 | +.59 | −.12 | +.30 | −1.45 |
| May | −1.15 | −1.69 | +.48 | −1.56 | +2.01 | +4.35 |
| June | −.53 | −.64 | +.32 | +.71 | −1.10 | −.47 |
| Seasonal average | +.76 | −.73 | +.37 | +.05 | −.21 | +.26 |

The season of 1924–25 was mild and dry, except for December and January, which were cold, with a good protection of snow. The precipitation was well distributed throughout the hibernation period, but the deficiency was too great for favorable hibernation. On the eastern slope (cage 7) 7.26 per cent and on the northern exposure (cage 5) 10.03 per cent of the beetles hibernated, indicating that the deficiency of moisture in the hibernation material was greater on the eastern than on the northern exposure.

During the season of 1925–26 the fall temperature was moderate with a slight deficiency in moisture. The winter was variable, with a good blanket of snow during the cold months and an excess of moisture in March. The spring was slightly above normal in precipitation, which stimulated emergence in June. The season as a whole was nearly normal and favorable for overwintering. In the cage on the eastern slope 36.24 per cent and in that on the northern slope 22.24 per cent of the insects overwintered, indicating that the northern exposure received a slight excess of moisture above the optimum.

The season of 1926–27 was the warmest on record, with a slight excess of precipitation. December was the wettest month and May the driest. The spring was hot and dry, which conditions increased

mortality on the eastern slopes and decreased the death rate on the northern. On the eastern exposure 16.22 per cent of the beetles hibernated as compared with 28.29 per cent on the northern exposure. The excess rainfall in June stimulated emergence and thus decreased the length of the hibernation period.

The season of 1927–28 was comparatively warm, with two winter months below normal in temperature and six of the nine months deficient in precipitation. January was warm, clear, and exceedingly dry, the driest on record. May was below normal in temperature, and above normal in precipitation. Snow fell during December, February, April, and May; the amount falling in May was the largest recorded for that month. (Table 8.) June was hot and dry, and emergence of the beetles was delayed until the July rains. The season as a whole was slightly above normal in temperature, with a small decrease in precipitation. On the eastern exposure 2.30 per cent of the beetles survived the winter, and on the northern exposure 0.75 per cent.

For the winter of 1928–29 October and April were above normal in temperature, October being one of the warmest on record. The other months were below normal in temperature, February being the coldest—in fact, it was the coldest February recorded. March was cold and wet, with a heavy, wet snowfall on the 27th and 28th. Five of the nine months had an excess of precipitation, that for May being the greatest on record. June was warm and dry, with a slight precipitation which occurred at the close of the month. Emergence from hibernation was delayed until the July rains. The season as a whole was cold with a slight excess of precipitation. It was unfavorable for hibernation. In the cage on the northern slope 2.95 per cent of the beetles survived as compared with 3.58 per cent on the eastern slope.

Figure 11 shows that the seasons of 1923–24, 1925–26, and 1926–27 were favorable for winter survival, that 1924–25 was fair in this respect, and that 1927–28 and 1928–29 were the least favorable. The seasonal average departures from normal temperature and precipitation, as given in Table 9, show no correlation with winter survival. They do, however, fall within the seasonal variations in survival for the good winters. The only significant relationship is shown between the comparatively uniform seasons of 1923–24 and 1924–25, the cool, damp winter being more favorable for survival than the warm, dry season.

### EFFECT OF A COVERING OF SNOW IN RELATION TO TEMPERATURE FLUCTUATIONS

Tables 8 and 9 show a great variation in monthly temperatures from season to season at Tajique. For example, the temperatures in January, 1926 and 1927, showed a variation of 10.7° F. The snowfall for these months (Table 8) was 24.6 and 3.0 inches, respectively. In 1925–26, 36.24 per cent of the beetles survived in cage 7, as compared with 16.22 per cent in the same cage in 1926–27. March in 1924 and 1925 varied 9.2° in temperature, with 19.7 and 0.5 inches, respectively, of snowfall. In cage 7, 23.54 per cent of the beetles survived in 1923–24 as compared with 7.26 per cent in 1924–25.

The mean temperature at Tajique for January, 1925, and for January, 1926, was 22.8° F., with 11.3 and 24.6 inches of snowfall,

and in 1924–25 and 1925–26, 7.26 and 36.24 per cent of the beetles, respectively, survived in cage 7. Of the beetles in cage 11 (at Estancia), 21.80 per cent survived during the winter of 1925–26, when the monthly mean temperature decreased to 18.8° for January with a 10-inch snowfall. The mean minimum temperature for this month was −2.1°. (Table 6.)

These data show that during the coldest months of the year the beetles are usually well protected by a blanket of snow and that when there is sufficient covering of snow very few are killed. The lowest temperature recorded in the last three seasons by distant thermographs measuring temperature of natural hibernation material of oak leaves and pine needles was 16° F. In the Southwest the coldest periods follow snowfalls and are accompanied by clear weather.

### PRECIPITATION FLUCTUATIONS

During the season of 1923–24, October, November, and December had an excess of precipitation of 1.34, 2.11, and 1.75 inches (Table 9), and during the season of 1925–26 March had an excess of 2.81 inches. In 1926–27 December had an excess of 2.29 inches. This excess precipitation during the months mentioned had little effect on mortality, which indicates that these are not the critical months in the hibernation of the beetle. During the season of 1923–24 April had an excess of 1.85 inches of moisture without any great effect on the mortality of beetles in hibernation. In 1927–28 May was apparently the critical month in the hibernation of the insect. The heavy snowfall during April and May (Table 8) was evidently the cause of the high mortality, especially that in May, when precipitation occurred on 14 days. This conclusion is supported by the fact that 4.92 per cent of the beetles survived in cage 6, from which precipitation was excluded after May 10, as compared with 2.30 per cent survival in cage 7 near by.

During the season of 1928–29 May was cool and the precipitation recorded was the greatest, indicating that this was the critical month of the season. In cage 7, 3.58 per cent of the beetles survived as compared with 1.52 per cent in cage 6. The greatest mortality must have occurred before May 10, when natural precipitation was excluded from cage 6. This indicates that heavy precipitation in the form of rain in May, 1929, was not so detrimental as the heavy snowfall in May, 1928. The critical period must therefore have been subsequent to the heavy snowfall on March 27 and 28. Following this period there were 10 days in which the mean temperature averaged 42.7° F. as compared with 45° for the 10-day period subsequent to the heavy snowfall on May 11 and 12, 1928. The weather conditions during these periods were very favorable for the fungus *Beauveria globulifera*, which caused high mortality among the hibernating beetles. Cage examination during the latter part of April, 1929, showed that many had died before that time.

Cage examinations during the period of hibernation showed that very few beetles are killed during complete dormancy in the natural hibernation zone and that the period of heavy mortality occurs with and immediately following the melting of the accumulated snow, when the beetles are becoming semiactive and are absorbing moisture. For example, during the 1925–26 season beetles in cage 8 were in fine

condition until the snow began to melt, and cage examination on April 8 indicated that a large percentage of them would survive. At this time the beetles were becoming semiactive, as five of them were up on the cage, evidently to avoid the excess moisture. After the snow began to melt on the northern slope, where this cage was located, the hibernation material was saturated with moisture for a long time. Later examinations showed that the beetles were being killed by *Beauveria globulifera*, and on May 9 very few live ones were found. Only 1.83 per cent survived. During the warm, dry spring of 1927, 10.32 per cent of the beetles survived in this cage, whereas in the wet springs of 1928 and 1929 only 1.80 and 0.04 per cent survived.

## SUMMARY AND CONCLUSIONS

The hibernation of the Mexican bean beetle in the Upper Sonoran, Transition, and Canadian Life Zones has been studied in relation to the plant associations found therein. In the fir-spruce association of the Canadian Zone only 42 insects survived out of a total of 38,500 placed in hibernation during the five seasons 1923–24 to 1928–29, inclusive. This survival occurred during an abnormally warm winter with nearly normal precipitation. In the ponderosa pine, or Transition, zone, 133,340 beetles were used in six years (1923–24 to 1928–29, inclusive), with a total survival of 13,114, or 9.84 per cent. In the Upper Sonoran Zone 44,500 beetles were employed, with a survival of 1,210 adults, or 2.72 per cent. Under natural conditions hibernation is confined to oak mottes found along canyon streams near the upper edge of this zone. The comparison of these results shows a consistent relationship between the zones and the percentage survival. The results indicate that the ponderosa pine forest zone is the natural hibernation quarters of the beetle in the Southwest and becomes more favorable when oak trees are present in the association.

The accumulation of dust, the character of the hibernation material, and snow coverage during subzero weather have been found to affect mortality in hibernation. Drainage is also an important factor in hibernation, mountainous or hilly country being more favorable for winter survival than a low, flat country when other conditions are similar.

Elevation considered alone seems to have no significance within the zone of successful hibernation. The determining factors at high elevations are temperature, precipitation, and exposure.

In the ponderosa-pine forest zone during winters followed by mild, dry springs, the largest percentage of beetles survived in cages on a northern exposure, whereas during winters followed by cold, wet springs survival was greatest on an eastern exposure. A sunny exposure may make hibernation possible in a location otherwise too cold or damp.

Winter temperature is an important limiting factor in the distribution of the Mexican bean beetle. Unless other factors are favorable, such as a covering of snow and the character of the hibernation material, the temperatures in many sections of the northern part of the United States and in Canada are rather low for successful hibernation.

Precipitation is a major factor in successful hibernation. As the temperature decreases, so does the moisture requirement or toleration

of the insect. The seasonal distribution of precipitation seems to be more important than the quantity. Spring is the critical period in hibernation. Rain is not so detrimental as snow in the spring, especially if the snow is wet and heavy.

The parasitic fungus *Beauveria globulifera* is capable of causing a high death rate among overwintering beetles. The optimum conditions for growth and reproduction of this fungus follow heavy snowfalls or damp, rainy weather in the spring when the mean temperature is between 42° and 45° F.

Very few beetles are killed during complete dormancy; the period of heavy mortality occurs when the beetles become semiactive.

# OLFACTORY RESPONSES OF BLOWFLIES, WITH AND WITHOUT ANTENNAE, IN A WOODEN OLFACTOMETER [1]

By N. E. McIndoo

*Senior Entomologist, Division of Insect Toxicology and Physiology, Bureau of Entomology, United States Department of Agriculture*

## INTRODUCTION

In 1924 the writer began a series of studies dealing with the tropisms of insects. Shortly after the work was started it became evident that if the fundamentals of attractants and repellents were to be studied, special apparatus for measuring or recording the responses of insects to these substances must be developed. Later the writer (*5*) [2] devised and perfected such an apparatus and called it an "insect olfactometer." This type of apparatus was suitable only for slowly crawling insects, however, such as the Colorado potato beetle. Since 1926 several other investigators have made and used other types of olfactometers, but most of these have proved unsatisfactory.

In the spring of 1930 the present study was begun at the laboratory of the Bureau of Entomology at Takoma Park, Md. House flies were tested in special wire-screen cages, and it was soon learned that the flies can readily distinguish differences not only between the four classes of substances that induce the four human attributes of taste but also between various sweet liquids. The method used was suitable for determining the food preferences of flies as it let them come in contact with the substances to be tested, but it was not suitable for studying the fundamentals of attractants and repellents. After much work a satisfactory olfactometer was devised. This apparatus is called the wooden olfactometer to distinguish it from the glass olfactometer described by the writer in 1926. All attempts to use a modified form of the olfactometer made and successfully used with the Natal fruit fly (*Ceratitis rubivora* Coq.) by Ripley and Hepburn (*7*) were unsatisfactory for obtaining positive results quickly.

The original plan was to make a special study of the house fly (*Musca domestica* L.), but as the blue-bottle blowfly (*Calliphora erythrocephala* Meig.),[3] the green-bottle blowfly (*Lucilia sericata* Meig.), and the black blowfly (*Phormia regina* Meig.) reacted more satisfactorily in this type of olfactometer, they were used almost exclusively. In fact, these three species of blowflies proved to be ideal insects for this particular study.

## METHOD OF REARING FLIES

Flies were reared in wire-screen cages (fig. 1), 12 inches square and 18 inches tall. Each cage had a drawer (*a*) 4 inches deep which was kept half full of moist sand. The flies were fed 10 per cent sugar water in small earthenware dishes (*b*), 3 inches in diameter and 1.25 inches

[1] Received for publication, Aug. 31, 1932; issued May, 1933.
[2] Reference is made by number (italic) to Literature Cited, p. 625.
[3] The blowflies were identified by J. M. Aldrich, of the U.S. National Museum.

deep, and to prevent the insects from drowning in the liquid a disk of perforated cork was allowed to float on the surface of the sugar water. The flies were given tap water periodically in similar containers. To induce oviposition the blowflies were given Hamburg steak (*c*) in other dishes and the house flies laid eggs on moist bran in the box where their larvae later pupated.

Steak, bearing fly eggs, was put in battery jars (fig. 2, *a*), 6.5 inches in diameter and 8 inches tall, which contained sand and a supply of meat (*b*). The jars were then covered with cheesecloth and put in a dark box, where the larvae developed and finally pupated in the sand. When the imagoes began to emerge, the jars were removed from the dark box to a light place and flytraps (*c*) were placed over them. If the flies emerged slowly they were fed sugar water on pieces of cotton, suspended from stoppers in the tops of the traps. Periodically the flies were transferred to the cages by inserting the flytraps into cylinders (fig. 1, *d*) of black cloth, which were fastened around large holes in the cages. By this method large supplies of flies of known age were obtained.

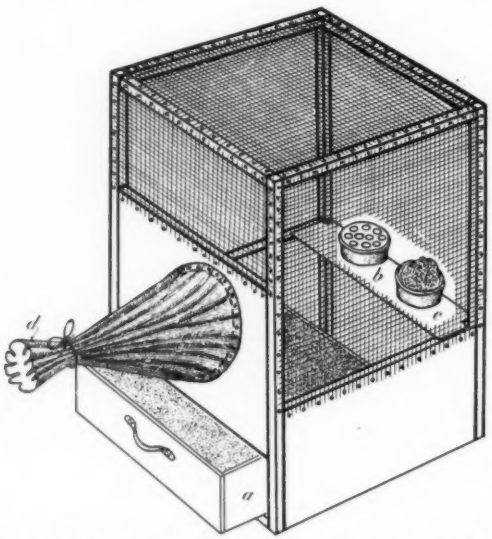


FIGURE 1.—Wire-screen cage in which flies were reared: *a*, Drawer half full of moist sand; *b*, small earthenware dish containing 10 per cent sugar water, on the surface of which floats a disk of perforated cork; *c*, earthenware dish containing Hamburg steak; *d*, black cloth cylinder used as an entrance and exit

When the weather was sufficiently warm the flies were reared on a south porch adjoining the writer's laboratory, but during the winter months the cages containing flies were kept on a temporary table built over a radiator by a west window of the laboratory. The battery jars containing larvae and foul-smelling meat were kept in a dark box in the basement near the furnace. Even without the aid of constant temperature and humidity chambers, a goodly supply of blowflies was usually to be had at any time. The only precautions taken were to observe the flies daily, chiefly to see that they had a sufficient quantity of fresh sugar water. Occasionally the cages were washed with water and the dead flies removed.

## THE DARK ROOM AND APPARATUS

A large closet, 6.5 feet wide, 7.5 feet long, and 9.5 feet tall, in the writer's laboratory was converted into an experimental dark room (fig. 3) by building a ventilator (*a*) in the window, suspending from

the ceiling a 60-watt light (*b*) inside a white globe, and making the door (*c*) light proof.

The olfactometer (*d*) stood on a table (*e*) near the center of the room directly beneath the light, which was 4 feet above. The three bottles were connected with a glass Y tube (*f*), which was attached to a long glass tube running to the wall of the room. The latter tube was connected with a small brass pipe that penetrated the wall and ran to the blower pump (*g*) outside the dark room. As this one-tenth horsepower pump made considerable noise, it was mounted on large rubber stoppers inside a box that had a lid. The box rested on the top of a bookcase that stood in the laboratory against the wall of the dark room. A 40-watt ruby light (*h*), when used, was suspended 1 foot directly above the olfactometer. A centigrade thermometer (*i*), the scale of which was marked in fifths of degrees, was hung on the wall. An electric fan (*j*) was used only during the hottest days because its air current interfered with the responses of the flies.

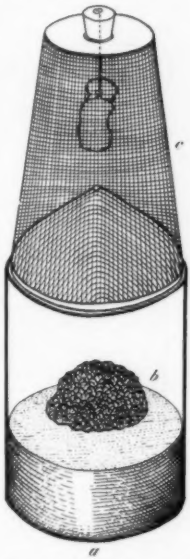


FIGURE 2.—Apparatus in which blowflies were reared and later were caught: *a*, Battery jar containing sand and Hamburg steak (*b*); *c*, flytrap from the top of which is suspended a piece of cotton wet with sugar water

## THE OLFACTOMETER

The wooden olfactometer (fig. 4), the simplest and most satisfactory one yet devised by the writer, consisted of two essential parts—a specially constructed wooden box and a set of three bottles with their connecting tubes. The box (*a*), 12 inches square and 3 inches deep (inside dimensions), had wooden sides and bottom and a wire-screen top that opened on hinges and was fastened with hooks.

The box was supported on four wooden legs 8.75 inches long. At one side of the box there was a stopper (*b*) closing a hole 1 inch in diameter. This hole was used as an entrance and exit when the flies were being transferred to and from the box. On the same side of the box there was a door (*c*), 2.5 inches long and 2 inches wide, through which the dead flies were removed and the glass dish for sugar water was introduced. This dish (*d*), 2 inches in diameter and 1 inch deep, usually contained fresh sugar water on which floated a cork disk, having several round holes through which the flies took food while standing on the cork. The most specialized part of the olfactometer was the arrangement of two cups (*e*) which disseminated the odors entering the box. The cups were carved from disks of soft pine, 1 inch thick and 3.5 inches in diameter, and when finished were coated with hard paraffin that masked the pine odor. The cups (*f*) were 2.25 inches in diameter and 0.5 inch deep, and the rim stood three-sixteenths inch above the level of the disk. Galvanized-iron disks, each bearing 50 small nail holes, served as covers for the cups. The cover was fastened firmly to the rim with two small nails. The rim of the cup fitted snugly into a hole in the bottom of the box so that the

perforated iron cover was flush with the inner surface of the bottom of the box. The cups were held in this hole by two rubber bands 1 inch wide, that were stretched the full width of the box on the under side. At the center of the bottom of the cup there was a hole just

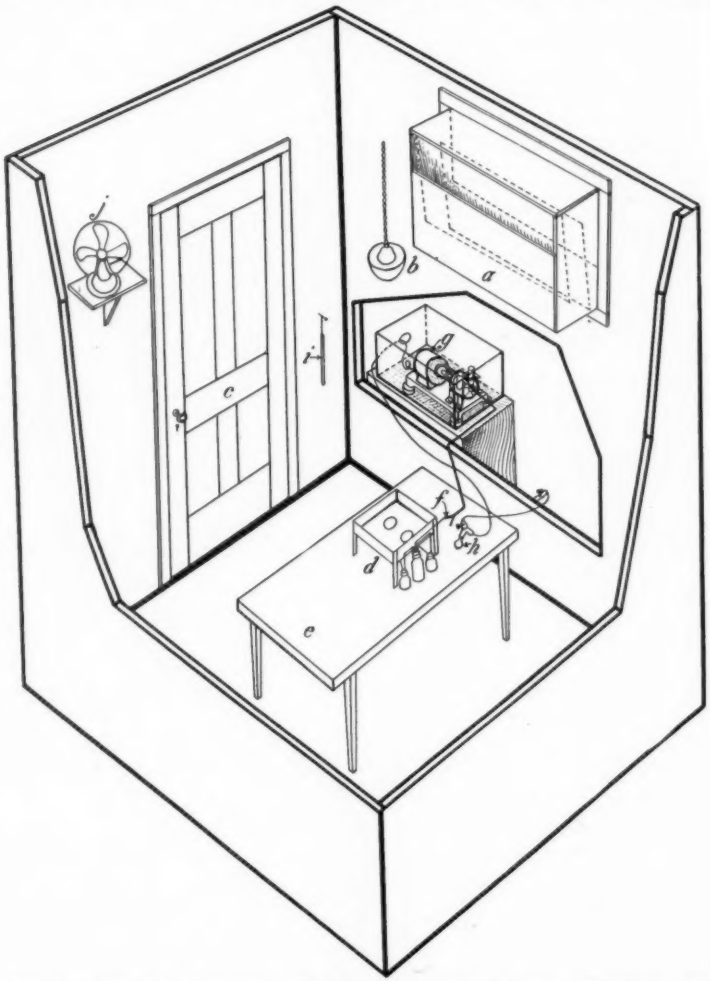


FIGURE 3.—Dark room and apparatus used in obtaining response of flies to odors: *a*, Ventilator; *b*, 60-watt light (not in correct position); *c*, door; *d*, olfactometer; *e*, table; *f*, glass Y tube; *g*, electric blower pump; *h*, 40-watt ruby light; *i*, centigrade thermometer; *j*, electric fan

large enough to admit the glass tube running from one of the smaller bottles.

The set of bottles consisted of one large bottle (fig. 4, *g*) of 200-cc capacity and two small ones (*h* and *i*) each of 100-cc capacity. Each bottle was closed with a No. 7 cork stopper that had been boiled in

hard paraffin. The stopper in the large bottle had three holes and each of the other stoppers had two. The glass tube (*j*), which connected bottle *h* with the nearer cup, was 7 inches long (not counting the two ends bent at right angles); while the corresponding tube (*k*), connecting bottle *i* with the farther cup, was 12 inches long. The tubes *l* and *m* were alike, each being 6 inches long. Tubes *n* and *o* were also alike, each being 4 inches long (not counting the two bent portions). The bore of each of the foregoing tubes was 3 mm. The length of tubes *p* and *q* was 6 inches each and the bore was 6 mm. The bore of the Y tube (*r*), of the glass tube (*s*), and of the brass tube which connected tube *s* with the blower pump was 8 mm. All of these tubes were securely united by short pieces of rubber tubing (*t*)

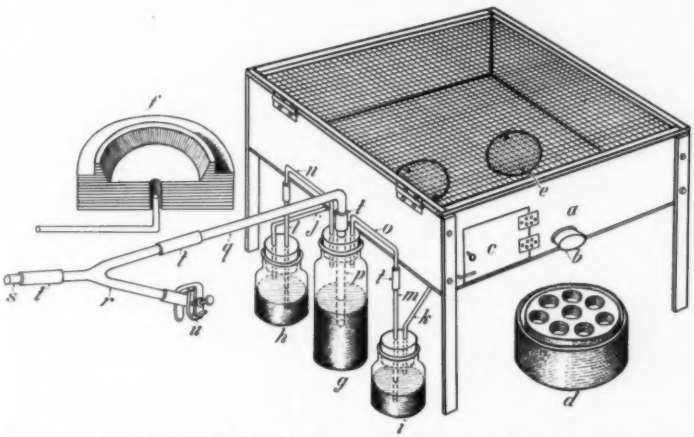


FIGURE 4.—Wooden olfactometer consisting of: *a*, Special wooden box in which flies were kept while being tested; *b*, stopper closing a hole used as an entrance and exit for flies; *c*, door of box through which dead flies were removed and through which sugar-water dish (*d*) was introduced when flies were not being tested, *e*, cup covered with perforated iron disk; *f*, portion of cup showing parts; *g*, large bottle; *h*, *i*, small bottles, each half full of liquid; *j*, *k*, tubes running from small bottles to cups; *l*, *m*, *n*, *o*, tubes connecting small bottles with large bottle; *p*, *q*, tubes connecting large bottle with Y tube (*r*), which unites with tube (*s*) running to blower pump; *t*, pieces of rubber tubing; *u*, clamp

in such a manner that practically none of the rubber was exposed to the air in the tube.

## TRANSFER OF FLIES AND METHOD OF OPERATING THE OLFACTOMETER

During the preliminary stage of this work only one olfactometer was used for both species of blowflies, but subsequently an olfactometer was used for each species. The following procedure was usually adhered to closely. If the flies were on hand each series of tests was started with a set of newly emerged flies that had eaten little or no food. The number in each set usually ranged from 200 to 300. If the flies were to be transferred from a trap (fig. 2, *c*) to the box of an olfactometer, the trap was covered with a black cloth and the flies were then attracted by light through the round opening in the top of the trap and into the olfactometer box through the hole (fig. 4, *b*). If they were to be transferred from a cage (fig. 1), they were caught quickly in large vials and transferred to the box by means of light

and without the aid of a black cloth. They were handled quickly, without being touched with the fingers or tools, and for two or three weeks, or until the close of the experiments, they remained in the box, where they were usually fed lightly each night with fresh sugar water.

On the day after the flies were placed in the box of an olfactometer, the empty sugar-water dish was removed and the insects were carried to the dark room and placed directly beneath the light. (Fig. 3.) After the bottles had been half filled with tap water, the olfactometer was assembled and connected with the blower pump, which was well oiled with white petrolatum oil. This oil has no noticeable odor, but air passing through the pump had a faint machinelike odor, which was practically removed by forcing the air through the water in the large bottle. (Fig. 4, *g*.) A uniform flow of air was maintained by the use of a clamp (*u*) on a rubber tube attached to one fork of the Y tube. The number of bubbles counted in a small bottle was about 180 per minute. This weak air current could not be perceived by the fingers but it could be felt by placing one's cheek almost against the disk.

Before the pump was started the flies were fairly well distributed throughout the box, but just as soon as the humid air current entered the box those nearest the disk perceived it and went to the disks. This fact explains why this type of olfactometer is ideal for certain flies, particularly blowflies. So far, however, it has not been found satisfactory for the Colorado potato beetle (*Leptinotarsa decemlineata* Say), the Mexican bean beetle (*Epilachna corrupta* Muls.), or the codling moth (*Carpocapsa pomonella* L.). House flies responded readily to the humid air current, but, owing to their extreme restlessness, it was difficult to obtain consistent results. When attractive odors were being tested the response to weak air currents was not necessary, but when repellent odors were tested it was all important, for without it no results could be obtained. As the flies soon failed to respond to air currents or to odors carried by these currents, 10 counts were taken at 15-second intervals. The flies counted were those on the disks and those around the edges not more than one-fourth inch away. The counts were begun a few seconds after the air was turned on. The sum of the 10 counts for one disk was considered the result of a single test. A calculating machine was used for making the calculations.

The following data illustrate the preceding points and others not yet mentioned:

On March 13, 1931, there were about 300 females of Lucilia in the box. They had been fed lightly the night before, and at 9 a. m. the dish containing sugar water was removed. At 1 p. m., when the testing was begun, the temperature of the room was 23° C. and that of the water in the three bottles was 21°. The first control test gave the following figures, which represent the number of flies on each of the disks (fig. 4), as determined by counts made at 15-second intervals:

Humid air from bottle *h*—16+18+16+15+16+13+15+17+14+13=153.
Humid air from bottle *i*—18+16+13+13+13+14+17+16+15+12=147.

These figures show that the difference between any two counts on the same disk was usually not great. After bottles *h* and *i* had been interchanged, humid air from bottle *h* attracted 137 flies and that from bottle *i*, 117. In the first control test, bottle *h*=51 per cent attractive; in the second control test, bottle *h*=53.9 per cent attractive; average attractiveness, 52.4 per cent.

The water was removed from bottle *h* and an equal quantity of fermented granulated-sugar water was put in it. With the same procedure, the two tests gave the following results: Sugar water attracted 200 and 175 flies, respectively, and control water attracted 119 and 96 flies, respectively. In the first test of sugar water it was 62.7 per cent attractive; in the second test it was 64.6 per cent attractive; average attractiveness, 63.6 per cent.

Five days later a mixture of very putrid Hamburg steak and water was boiled hard for an hour and some of the boiled liquid was tested, the flies from the preceding test being used. The two tests gave the following results: Control water attracted 149 and 160 flies, respectively, and foul liquid attracted 50 and 60 flies, respectively. The repellency was obtained by calculating the attractiveness of the water as control. The first test of foul liquid was 74.9 per cent repellent; the second test, 72.7 per cent repellent: average repellency, 73.8 per cent.

After using about 300 males and females of Calliphora and testing other substances, it was ascertained that these flies, if fed lightly the night before, did not respond in large numbers; but if given neither food nor water overnight they responded in greater numbers, although the final percentages were not usually changed. A single test illustrates this point:

Water as control—4+5+3+2+3+2+3+3+0+1=26.
Water from sour milk—20+20+25+25+25+25+25+25+25+25=240.

The sour milk was 90.2 percent attractive.

When the flies were satiated with water or sugar water it was useless to carry on experiments. They did not then respond to most of the test liquids in sufficient numbers to give reliable results, but when certain much more attractive odors were used the factor of water satiation did not materially interfere with the responses expected. The plan finally adopted was to feed the flies lightly the night before the test and not to start the test until 10 or 11 a. m. This procedure was not at all necessary, however, when testing the odors of foul meat and a few other highly attractive materials.

Flies responding to the control liquid were in most instances accurately counted; but when responding to humid air or to odors in numbers more than 12 or 14, they were estimated, for it was impossible to count them, owing to their restlessness. Many times they were estimated, then counted, and the estimated number was nearly always smaller than the actual one.

It has already been shown that in running control tests the numbers of flies on the right and left disks did not differ materially. This was true only when the olfactometer was in perfect working order. If one cup or disk were scented or if a drop of odorous liquid were drawn into a cup, the numbers differed widely. The cups and disks were ordinarily made odorless by running air through them. Often they were removed and washed with water; but after foul odors had been tested, they were washed with alcohol and well aired. Before a series of tests was started the olfactometer was put in perfect working order and thereafter no liquid was permitted to come in contact with the cups or disks, which were cleaned and used in such a way that little or no error resulted from using the same cups repeatedly.

In a few tests the control response was actually 50 per cent. In most tests it was not above 52 or below 48 per cent, and the highest

recorded was 54.8 per cent. Responses above 50 per cent, not preceded by a plus or a minus sign, will hereafter be called attractive; while those above 50 per cent, preceded by a minus sign, will be called repellent. Nevertheless, in each test 2 or 3 per cent should be deducted as the probable error.

The flies, particularly the Lucilia, were sensitive to dry-air currents, but less so than to humid-air currents. Dry-air currents were not used as controls for odor responses because only odorous liquids were tested.

Flies were found sensitive to warm humid-air currents when cool humid air was used as a control. When there was only 2° or 3° difference in temperature between the water in the small bottles little or no difference in response was observed; but in one instance where there was a difference of 9° the warmer current was 74.4 per cent attractive. Similar results were obtained when dry currents and warm humid currents were tested. When humid currents were compared with dry currents the former were always the attractive ones. When humid air was forced through only one cup the response was as high as 85.8 per cent; the other 14.2 per cent of the flies just happened to be above the other cup, which was not a control and not connected with the other bottle.

After the foregoing results were obtained, the temperatures of the liquids in all three bottles were equalized by the use of cold and hot water and the thermometer brought from the dark room. The temperatures in most tests were near that of the laboratory.

## DIFFERENCES IN RESPONSE OF MALE AND FEMALE BLOWFLIES

In order to obtain accurate results for comparative purposes several factors were considered during the preliminary stage of this work; however, since no long series of tests was specially planned and conducted in order to furnish definite information on all of these factors, the following data sufficed as a foundation for more thorough experimentation.

Of all the factors considered, the state of water satiation seemed to be the only one that interfered with the testing. This factor was easily controlled, as already stated, by giving the flies a small quantity of water or sugar water overnight. Other factors were considered, but no important differences in response were obtained that could be attributed to differences in age, in sex, or even in sexual maturity of the males and females tested.

Some of the results obtained between February 11 and March 11 varied considerably. To determine whether this variation was due to different proportions of males and females in the same box, the sexes of 599 Lucilia were separated on March 11 and 12, when the flies were 13 days old. Separation was easily effected by catching the flies in large vials, in which they were chilled, either out of doors or in an electric refrigerator. While their compound eyes were being observed, they were kept in cold water. In all, there were 219 males and 380 (or 63.4 per cent) females. Next, all the dead flies in the cage—47 females and 151 males—were examined. At the time of emergence, therefore, the females predominated slightly, constituting 53.6 per cent of the total number. The males were usually smaller and less hardy than the females, and died more quickly. On April 7, 574 Calliphora,

4 days old, were separated on the basis of sex, and 304, or 53.0 per cent, were found to be males.

The Lucilia were tested from March 12 to March 18 and the Calliphora from April 8 to April 14. Unfortunately, some of the materials tested, particularly the fermented ones, were slowly changing, but at that time of year the chemical changes taking place in them usually were not great. As not all the experimental conditions were exactly alike the figures in Table 1 should not be compared too closely. The chief purpose in submitting these figures is to show that there were few wide differences in response and that the averages for the males and females of the same species were practically the same.

TABLE 1.—*Olfactory responses of male and female blowflies to various substances*

| Materials tested | Lucilia | | Calliphora | |
|---|---|---|---|---|
| | Males | Females | Males | Females |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| Control (water on each side) | 51.8 | 51.0 | 50.3 | 52.0 |
| Fermented granulated-sugar water | 56.1 | 64.6 | 69.3 | 74.6 |
| Fermented granulated-sugar water and yeast | 54.2 | 76.9 | 70.0 | 69.8 |
| Fermented brown-sugar water | 73.2 | 64.5 | 81.9 | 79.7 |
| Fermented brown-sugar water and yeast | [a] 56.0 | 61.3 | 83.2 | 66.7 |
| Fermented yeast mixture | 83.9 | 85.2 | 86.7 | 70.3 |
| Alcohol (about 6 per cent) | [a] −51.7 | 52.4 | 74.0 | 77.5 |
| Fresh milk | 72.9 | 55.6 | 67.9 | 66.7 |
| Water from clabbered milk | 64.1 | 78.6 | 77.8 | 77.9 |
| Fermented lactose mixture | 60.1 | 61.3 | 93.0 | 92.3 |
| Lactic acid (10.2 per cent) | 74.5 | 67.0 | 72.5 | 78.6 |
| Fermented casein mixture | 71.3 | 62.3 | 83.6 | 94.6 |
| Formalin (1 cc of formaldehyde to 60 cc of water) | 74.0 | 61.7 | 61.5 | 70.0 |
| Fermented molasses and yeast | 54.5 | 60.7 | 74.5 | 60.9 |
| Water from putrid Hamburg steak | 84.6 | 82.3 | 89.4 | 90.5 |
| Fermented molasses | 60.9 | 66.0 | | |
| Fermented corn sirup | 60.1 | 69.9 | | |
| Ammonia water (0.05 cc to 60 cc of water) | | | 66.7 | 71.0 |
| Stale urine | | | 76.1 | 64.6 |
| Stale feces | | | 80.0 | 63.7 |
| Stale distillate from water and putrid steak | | | 87.6 | 82.1 |
| Skatol (water moderately scented) | | | 54.5 | 55.0 |
| Average | 64.5 | 65.9 | 75.0 | 72.9 |

[a] For negative numbers the complement was used in computing the average, e. g., 100−56=44.

## RESPONSE TO ODORS FROM FERMENTING AND PUTREFYING SUBSTANCES

In an attempt to obtain fundamental facts concerning the attraction of insects to odors from various fermenting and putrefying substances much experimental work was done. In these experiments the following materials were tested: 10 per cent solutions of granulated and brown sugars, with and without the addition of baker's yeast; various concentrations of carbon dioxide, alcohol, and acetic acid; milk and three of its constituents (lactic acid, lactose, and casein); putrid Hamburg steak, putrid eggs, human feces, human urine, ammonia, and skatol. The absence of accurate knowledge of the chemical composition of the various substances made it impossible to obtain more than a limited amount of definite information on the attractants concerned in the tests. Hence, as to this, only a brief summary is given in the present paper.

Sugar solutions not containing baker's yeast were attractive up to the seventeenth day, being most attractive (76.2 per cent) on the

fourth day. Between the eighteenth and twenty-fifth days (end of tests) they were repellent, being most so (72 per cent) on the last day they were tested. Sugar solutions containing baker's yeast were attractive only up to the twentieth hour, being most attractive (66.7 per cent) one hour after they were prepared. They were most repellent on the third day (93.7 per cent) and on the twenty-first day (94.6 per cent), but on the twelfth day they were only slightly repellent (55.9 per cent). Curves representing the attractiveness of these materials would therefore have two ascents and two descents. All concentrations of the carbon dioxide and acetic acid tested were repellent. All concentrations of the alcohol up to 6 per cent, inclusive, were attractive, but the higher concentrations were repellent. The 4 per cent concentration was most attractive (67.8 per cent), and the 20 per cent concentration most repellent (96.2 per cent). The evolution of carbon dioxide and the production of alcohol and acetic acid in the fermenting mixtures apparently had much to do with the responses obtained.

The only pertinent reference to the fermenting mixtures mentioned here is by Eyer and Rhodes (*1, p. 702*). They state:

> Chemical analyses of codling moth (*Carpocapsa pomonella*) molasses baits during their periods of maximum attractiveness reveals their attrahent value to be most closely associated with certain changes in the decomposition of the sugars which precedes the formation of alcohol and acetic acid and is first evidenced by a decrease in the glucose content of the baits. These phenomena which are probably attended by the formation of esters, the exact composition of each of which is as yet undetermined, are of primary importance. The formation of alcohol and the evolution of gas are important secondary factors, while the production of high yeast populations and the conversion of alcohol into acetic acid are only slightly attractive or actually repellent. Under New Mexico conditions, where the average mean daily temperature for the period during which the Codling moth adults are most active (May 1 to September 30) is 73.5° Fahrenheit, the effectiveness of molasses baits is actually decreased by adding yeast in commercial form while on the other hand it is materially increased and the period of attractiveness prolonged by the addition of certain preservatives, particularly Benzoate of Soda which delays fermentation.

Milk and its constituents—lactic acid, lactose, and casein—were always attractive. The more sour the milk the more attractive it was, the lactic acid in the milk probably being its chief attracting ingredient. Fermented casein and baker's yeast were among the best attractants, but putrid meat and putrid eggs were the most attractive. The responses to odors from such putrid materials were apparently caused by a "desire" to oviposit rather than to feed but, strange to say, the males responded as well to such odors as did the females. Although the sense of smell in blowflies is in many respects similar to that in human beings, the writer found it to be more acute than his own.

According to the average percentages (Table 1) obtained by testing fermented yeast mixture (81.5 per cent), fermented lactose mixture (76.7 per cent), fermented casein mixture (77.9 per cent), and water from putrid Hamburg steak (86.7 per cent) the attractiveness of the putrid steak appears to be little greater than that of each of the other three substances named. However, the difference is greater than it appears, for the total number of flies estimated to be on the disk is not a fair criterion by which to judge the attractiveness of foul-meat odors.

During the first three estimations nearly half of the flies in the box were attracted toward the left disk, but not more than 60 at a time

were recorded as being attracted. They piled upon one another on the disk two or three layers deep, and for about 3 inches around the margin they congregated, all headed toward the disk, waiting to climb upon the others. In a single test 395 flies responded to water from foul meat on the left while 85 responded to water as control on the right. The foul odor was, therefore, 82.3 per cent attractive. The number of flies responding on the control side was unusually large, and this accounts for the comparatively low percentage of attractiveness.

## TESTS TO DETERMINE WHETHER BLOWFLIES SMELL WITH THEIR ANTENNAE

Before 1880 there was a controversy about the location of the olfactory organs in insects. In that year Hauser (*3*) apparently settled the matter by proving that the organs of smell are located in the antennae. In 1914 McIndoo (*4, p. 333–341*) convinced himself that honeybees do not smell with their antennae, but with widely scattered structures called olfactory pores. Von Frisch (*2*) in 1921 convinced most of the critics that honeybees do smell with their antennae. In 1924 Minnich (*6*) proved that nearly half of the olfactory organs of the cabbage butterfly (*Ascia rapae* L.) must be located elsewhere than in the antennae. With blowflies of the two larger genera, Calliphora and Phormia, and with the aid of the wooden olfactometer, the writer decided to close the controversy, if possible, so far as blowflies are concerned.

On January 1 and 2, 1932, 315 Calliphora emerged, 56.2 per cent being females. On January 4 and 5, the antennae or parts of antennae of 283 flies (126 males and 157 females) were pulled off, the following technic being used: About 30 flies at a time were caught in a large vial which was then placed in ice water. After the flies had become chilled the inactive ones were held between two fingers under a dissecting lens and the antennae were pulled off with fine-pointed forceps. The flies were then placed in the box of the olfactometer, where they were fed lightly over night and where they remained until they died. These mutilated flies could still fly well and responded normally to light and to odors, but they were comparatively inactive, both inside and outside the box, and were therefore easily caught with the fingers. They lived from 1 to 16 days, or an average of 8.2 days.

An excised antenna or part of one, when carefully examined under a high-power lens, was seen to have 1, 2, or 3 segments, but the third, or terminal segment, which bears the so-called olfactory hairs, was always present. Therefore all of the third segments were prevented from functioning when the insects with removed antennae were tested, but 17.1 per cent (18.7 per cent in males and 15.6 per cent in females) of the total number of second segments and 49.2 per cent (52.6 per cent in males and 45.9 per cent in females) of the total number of first segments remained on the heads.

Since there were not enough unmutilated Calliphora for a second set of flies, the responses of the mutilated ones which were tested from January 6 to 14, 1932, were compared with those of a set of unmutilated Calliphora tested in the summer of 1931. (Table 2.) Although the nine substances tested in 1931 and in 1932 were not exactly identical, the average responses (76.1 and 75.5 per cent) of the two sets of flies were practically the same.

TABLE 2.—*Responses of Calliphora with and without antennae to various odors*

| Substance tested | Unmutilated flies | Flies with antennae pulled off |
|---|---|---|
| | *Per cent* | *Per cent* |
| Fresh milk | 69.2 | 59.8 |
| Sour milk | 77.3 | 86.5 |
| Lactic acid (10.2 per cent) | 76.2 | 84.5 |
| Alcohol (4 per cent) | 77.6 | 74.8 |
| Formalin (1 cc to 60 cc of water) | 65.7 | 60.0 |
| Brown-sugar water | 78.9 | 78.0 |
| Water from putrid Hamburg steak | 89.9 | 88.7 |
| Stale urine | 76.1 | 75.3 |
| Stale distillate from putrid steak | 74.5 | 72.3 |
| Average | 76.1 | 75.5 |

The next series of experiments, extending over a period of eight days, consisted of testing milk, brown-sugar water (10 per cent concentration), five concentrations of alcohol, and seven concentrations of lactic acid. The milk and sugar water were tested when fresh (Table 3) and then put in pint milk bottles. The bottles were covered with cheesecloth and placed in the laboratory. The concentrations of alcohol and lactic acid were prepared by using absolute alcohol, 85 per cent lactic acid (U. S. P.), and distilled water. The milk and sugar water were tested only once daily, but four tests for each concentration of alcohol and lactic acid were usually conducted. The average percentages of attractiveness of these substances are shown in Tables 3 to 5.

TABLE 3.—*Responses of Calliphora with and without antennae to odors from milk and brown-sugar water*

| Age of material (days) | Milk | | Brown-sugar water | |
|---|---|---|---|---|
| | Unmutilated flies | Flies with antennae pulled off | Unmutilated flies | Flies with antennae pulled off |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 0 | 69.2 | 59.8 | 64.1 | 64.3 |
| 1 | 77.3 | 71.4 | 67.3 | 66.3 |
| 2 | 72.5 | 74.4 | 67.4 | 67.3 |
| 3 | 75.7 | ---- | ---- | 70.3 |
| 4 | 73.3 | 75.4 | 78.9 | 78.0 |
| 6 | 67.8 | 73.1 | 72.1 | 70.2 |
| 7 | 66.1 | 68.4 | ---- | ---- |
| 8 | 62.8 | 60.0 | 71.7 | ---- |
| Average | 70.6 | 68.9 | 70.2 | 69.4 |

Curves representing the foregoing responses of the unmutilated and mutilated flies closely follow each other. They and the average percentages given in Tables 2 to 5 show clearly that Calliphora do not smell with their antennae.

The experiments were repeated, using Phormia, and this time more care was exercised in pulling off the antennae. On January 13 and 14 more than 600 Phormia emerged, and on January 15 and 16 the antennae of 300 (113 males and 187 females) were removed under a binocular microscope. The mutilated insects were then placed in one box of the olfactometer, and 300 unmutilated flies, in approximately equal numbers of males and females, were placed in another

box. At each wound a tiny droplet of blood issued, but the flies apparently did not suffer and they appeared normal in nearly all respects. They were less active than usual, however, and were easily caught with the fingers. They mated readily and continued to lay eggs up to the time of their deaths. The mutilated flies began ovipositing two days later than the unmutilated flies and deposited only about one half as many egg masses.

TABLE 4.—*Responses of Calliphora with and without antennae to odors from five concentrations of alcohol*

| Concentration of alcohol (per cent) | Unmutilated flies | | Flies with antennae pulled off | |
|---|---|---|---|---|
| | Individual tests | Average for four tests | Individual tests | Average for four tests |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 1 | 59.8 | 60.30 | 56.8 | 61.30 |
| | 60.8 | | 65.0 | |
| | 58.6 | | 63.9 | |
| | 62.0 | | 59.4 | |
| 2 | 62.5 | 65.75 | 61.1 | 63.70 |
| | 72.3 | | 63.4 | |
| | 68.7 | | 61.5 | |
| | 59.5 | | 68.7 | |
| 4 | 77.6 | 72.00 | 67.8 | 72.40 |
| | 68.9 | | 73.8 | |
| | 69.7 | | 73.1 | |
| | 71.8 | | 74.8 | |
| 6 | 55.3 | 60.25 | 60.5 | 62.70 |
| | 61.7 | | 66.1 | |
| | 60.6 | | 63.6 | |
| | 63.4 | | 60.7 | |
| 8 | 55.4 | 52.12 | 51.8 | 52.95 |
| | 53.4 | | 53.8 | |
| | 51.7 | | 56.2 | |
| | [a] −52.0 | | 50.0 | |
| Average | 62.12 | 62.08 | 59.60 | 62.60 |
| | 63.42 | | 64.42 | |
| | 61.86 | | 63.66 | |
| | 60.94 | | 62.72 | |

[a] Complement of this number was used in obtaining average.

The removed antennae were mounted in pairs in glycerin on two slides and the segments in each were counted. The third or terminal segment was always pulled off, the second segment was usually severed, but the minute first segment was often left attached to the head. Of the males, 38.0 per cent had been deprived of all 6 segments; 26.5 per cent of 5 segments; 24.8 per cent of 4 segments; 4.5 per cent of 3 segments; and 6.2 per cent of only 2 segments. Of the females, 40.7 per cent had been deprived of all 6 segments; 23.0 per cent of 5 segments; 25.0 per cent of 4 segments; 7.5 per cent of 3 segments; and only 3.8 per cent of 2 segments. An average of 39.3 per cent of both males and females had been deprived of all 6 segments; 24.7 per cent of 5 segments; 24.9 per cent of 4 segments; 6.0 per cent of 3 segments; and only 5.0 per cent of 2 segments.

As each box contained a larger number of flies, it was impossible to obtain complete records of individuals; therefore those that had been deprived of all or nearly all of their antennal segments might not have responded at all. Having failed to obtain satisfactory records by using only 1, 2, or 3 mutilated Calliphora at a time in the box, 7 active unmutilated Phormia were placed in one box and 7 active

mutilated Phormia in another, each fly in a box being distinctively marked with white ink for easy identification. On a form used in tabulating the results the symbol for each fly indicated the sex of the insect and the part or parts of its anatomy that were marked. The following abbreviations were used: t for thorax; r for right wing; l for left wing; and a for abdomen. The unmutilated flies were, therefore, given the following symbols: t♂, r♀, l♂, rl♂, tr♀, tl♂, and a♀. The symbols of the mutilated flies were t♀, r♀, l♂, rl♂, tr♀, tl♂, and a♀. The marked flies were among a large number of unmarked flies.

TABLE 5.—*Responses of Calliphora with and without antennae to odors from seven concentrations of lactic acid*

| Concentration of lactic acid (per cent) | Unmutilated flies | Flies with antennae pulled off | |
|---|---|---|---|
| | | Individual tests | Average for four tests |
| | *Per cent* | *Per cent* | *Per cent* |
| 1.7 | 53.6 | 54.4<br>60.0<br>59.1<br>60.6 | 58.5 |
| 3.4 | 59.5 | 61.4<br>63.7<br>61.6<br>58.6 | 61.3 |
| 6.8 | 72.7 | 67.0<br>67.0<br>71.0<br>69.3 | 68.6 |
| 10.2 | 76.2 | 71.9<br>72.1<br>76.6<br>84.5 | 76.3 |
| 13.6 | 73.7 | 60.0<br>73.7<br>73.3<br>67.8 | 68.7 |
| 17.0 | 60.0 | 58.1<br>62.7<br>64.2<br>67.2 | 63.0 |
| 25.5 | [a]—53.6 | 51.3<br>58.1<br>57.0<br>57.9 | 56.1 |
| Average | 63.15 | 60.58<br>65.32<br>66.11<br>66.55 | 64.64 |

[a] Complement of this number was used in obtaining average.

Fresh brown-sugar water (10 per cent concentration), fresh milk, concentrations of alcohol and lactic acid, and mixtures containing baker's yeast cake (3 g in 250 cc of water), and purified casein (3 g in 250 cc of water) were prepared. Pint milk bottles containing the sugar water, milk, yeast mixture, and casein mixture were covered with cheesecloth and placed in the laboratory. The alcohol and lactic acid were kept in well-stoppered bottles. The tests were run from January 18 to 26.

Tables 6 to 8 show the average percentages of attractiveness of the substances. The general average is, for unmutilated flies, 66.6; for mutilated flies, 67.4. Again, it is clearly shown that blowflies do not smell with their antennae.

TABLE 6.—*Responses of Phormia with and without antennae to odors from brown-sugar water, milk, yeast mixture, and casein mixture*

| Age of material (days) | Brown-sugar water | | Milk | | Yeast mixture | | Casein mixture | |
|---|---|---|---|---|---|---|---|---|
| | Unmutilated flies | Flies with antennae pulled off | Unmutilated flies | Flies with antennae pulled off | Unmutilated flies | Flies with antennae pulled off | Unmutilated flies | Flies with antennae pulled off |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 0 | 64.3 | 65.1 | 59.3 | 64.3 | 65.2 | 70.4 | 63.6 | 67.1 |
| 1 | 68.0 | 72.1 | 66.2 | 69.8 | 65.9 | 68.5 | 69.0 | 67.2 |
| 2 | 68.2 | 69.6 | 66.0 | 71.7 | 68.4 | 71.4 | 68.5 | 72.5 |
| 3 | 69.3 | 69.8 | 78.0 | 72.7 | 67.4 | 70.4 | 69.7 | 67.1 |
| 4 | 73.6 | 75.3 | 79.8 | 76.1 | 77.4 | 73.8 | 76.4 | 80.3 |
| 5 | 65.4 | 65.2 | 66.0 | 68.4 | 72.0 | 65.5 | 77.7 | 75.8 |
| 7 | 57.6 | 60.2 | 62.3 | 60.2 | 71.2 | 75.4 | 81.2 | 74.3 |
| 8 | 57.4 | 60.0 | 59.2 | 60.9 | 72.2 | 76.1 | 80.8 | 81.6 |
| Average | 65.5 | 67.2 | 67.1 | 68.0 | 70.0 | 71.4 | 73.4 | 73.2 |

TABLE 7.—*Responses of Phormia with and without antennae to odors from five concentrations of alcohol*

| Concentration of alcohol (per cent) | Unmutilated flies | | Flies with antennae pulled off | |
|---|---|---|---|---|
| | Individual tests | Average for 4 tests | Individual tests | Average for 4 tests |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 1 | 57.3<br>59.4<br>58.6<br>58.2 | 58.375 | 56.6<br>59.5<br>58.5<br>58.8 | 58.35 |
| 2 | 62.6<br>61.8<br>66.1<br>64.9 | 63.85 | 62.9<br>62.9<br>67.3<br>63.5 | 64.15 |
| 4 | 73.7<br>65.4<br>65.9<br>66.2 | 67.80 | 74.4<br>65.4<br>70.0<br>66.1 | 68.975 |
| 6 | 63.4<br>63.9<br>62.5<br>60.0 | 62.45 | 64.1<br>62.5<br>63.5<br>55.8 | 61.475 |
| 8 | [a] 50.7<br>[b] −56.9<br>[b] −58.5<br>[b] −54.6 | −54.825 | [a] 50.9<br>[b] −51.5<br>[b] −50.6<br>[b] −59.0 | −52.55 |
| Average | 61.54<br>58.72<br>58.92<br>58.94 | 59.53 | 61.78<br>59.76<br>61.74<br>57.04 | 60.08 |

[a] Complement of this number was used in obtaining average, 4 tests, but the percentage as it stands was used in obtaining average, first test, all concentrations.

[b] Percentage as given was used in obtaining average concentration 8, but its complement in obtaining averages of the second, third, and fourth tests, all concentrations.

On the basis of equal response by all the flies in the box the data for 4 days showed more than proportional response by the marked flies and the mutilated flies responded slightly better than did the unmutilated ones. The females responded almost as often as the males. The total number of responses of each of the 14 marked flies ranged from 3 to 24, with an average of 13.9. Considering all of the 14 marked flies and the 7 substances tested, water (as a control) offered 50 per cent of all the chances to respond, while each of the other 6 materials offered only 8.3 per cent. However, the following

data clearly show that the number of responses were not so divided. Water induced only 21.6 per cent of all responses; brown-sugar water, 9.8 per cent; casein solution, 9.3 per cent; lactic acid, 19.6 per cent; yeast mixture, 9.8 per cent; alcohol, 10.8 per cent; and milk, 19.1 per cent. Of the 7 mutilated flies alone, water induced only 18.7 (and not 50) per cent of all the responses; brown-sugar water, 5.6 per cent; casein mixture, 7.5 per cent; lactic acid, 18.7 per cent; yeast mixture, 13.1 per cent; alcohol, 14.0 per cent; and milk, 22.4 per cent. The total number of responses to all of the 7 materials tested is as follows: t♀, 8; r♀, 16; l♂, 16; rl♂, 20; tr♀, 24; tl♂, 15; and a♀, 8. When these 7 mutilated flies had died, their heads were examined to determine how many antennal segments remained attached. This was done by teasing out the "stumps" of the antennae with needles under a binocular microscope. The following results were noted: tl♂ bore no antennal segments; r♀, l♂, and rl♂ each bore one segment; t♀ and tr♀ each bore two first segments; and a♀ bore two first segments and one second segment. The 7 mutilated flies lived from 11 to 58 days, or an average of 31.6 days, and the 7 unmutilated flies lived from 1 to 58 days, or an average of 26 days. The 300 mutilated flies lived from 1 to 59 days, or an average of 15.9 days; the 300 unmutilated flies lived from 1 to 60 days, or an average of 18.2 days.

TABLE 8.—*Responses of Phormia with and without antennae to odors from seven concentrations of lactic acid*

| Concentration of lactic acid (per cent) | Unmutilated flies | | Flies with antennae pulled off | |
|---|---|---|---|---|
| | Individual tests | Average for 4 tests | Individual tests | Average for 4 tests |
| | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| 1.7 | 55.8<br>58.2<br>59.1<br>57.6 | 57.675 | 59.1<br>58.3<br>58.1<br>58.0 | 58.375 |
| 3.4 | 64.7<br>59.6<br>58.6<br>65.7 | 62.15 | 65.4<br>56.9<br>62.8<br>69.0 | 63.525 |
| 6.8 | 69.0<br>62.6<br>63.5<br>76.9 | 68.0 | 73.8<br>68.6<br>64.0<br>66.0 | 68.10 |
| 10.2 | 69.8<br>66.7<br>68.4<br>72.6 | 69.375 | 70.8<br>69.3<br>70.7<br>76.5 | 71.825 |
| 13.6 | 66.7<br>60.0<br>62.0<br>74.8 | 65.875 | 72.5<br>65.5<br>67.5<br>70.1 | 68.90 |
| 17.0 | 62.1<br>59.0<br>62.8<br>66.3 | 62.55 | 63.8<br>62.7<br>63.1<br>62.2 | 62.95 |
| 25.5 | 60.8<br>58.9<br>60.6<br>68.7 | 62.25 | 59.1<br>59.2<br>61.9<br>58.8 | 59.75 |
| Average | 64.13<br>60.71<br>62.14<br>68.94 | 63.98 | 66.35<br>62.93<br>64.01<br>65.80 | 64.77 |

Some of the experiments were again repeated, using Calliphora. On January 18 about 140 Calliphora emerged and on January 25 and

26 the antennae of 134 of them were pulled off. This time the insects were not chilled by using ice water and a greater effort than ever was made to pull off all the segments by seizing, if possible, the minute first segments with the needle-pointed forceps. These operations were fatal to many of the flies, 93 of them having died by January 27, when the survivors (13 males and 28 females) were tested. An examination of the heads of the dead flies showed that 41.4 per cent of them had been deprived of all six antennal segments. On the heads of the others 40.2 per cent of the first segments and 21.9 per cent of the second segments remained, but no third segments.

On January 27, between 12.30 and 1.20 p. m., 10 males and 18 females, the most active of the surviving flies, were subjected to another operation. This time, in order to eliminate the possibility that the remaining antennal segments or the exposed antennal nerves might still receive olfactory stimuli, tiny droplets of glue were placed on the "stumps" of the antennae, and again the flies were tested. (Table 9.) When these flies had died, it was observed that the glue remained where placed and only 37.5 per cent of the first segments were present on the heads. The 41 tested flies lived from 1 to 14 days, or an average of 3.8 days. The flies with glue on their heads lived an average of 4.2 days. The data in Table 9 again clearly show that the antennae of blowflies do not carry the olfactory organs.

TABLE 9.—*Responses of Calliphora with and without antennae to various odors*

| Substance tested | Unmutilated flies | Flies with antennae pulled off | Flies with antennae pulled off and stumps of antennae covered with glue |
|---|---|---|---|
| | *Per cent* | *Per cent* | *Per cent* |
| Brown-sugar water (9 days old) | 71.7 | 74.1 | 75 |
| Milk (9 days old) | 62.8 | 69.2 | 69 |
| Lactic acid (10.2 pe. cent) | 76.2 | 74.2 | 76 |
| Yeast mixture (9 days old) | 78.5 | 75.9 | 75 |
| Casein mixture (9 days old) | 89.1 | 81.2 | 75 |
| Average | 75.7 | 74.9 | 74 |

To obtain further information on this subject a careful study of the antennae of blowflies was made by using live antennae, caustic potash preparations, and many sections. The so-called olfactory hairs, lying in pits and pocket-shaped cavities in the third segments, are very numerous but they were not found elsewhere on the antennae. The first and second segments contain no sense organs of any kind, except the Johnston's organs in the second segment. These organs have no connection with the exterior and have never been regarded as olfactory organs. Furthermore, it was observed that wounds on the mutilated antennae soon healed, thereby preventing the air from entering at the places of injury.

## SUMMARY

In the hope of obtaining fundamental facts concerning the attraction of insects to odors from fermenting and putrefying substances, much experimental work was done with three species of blowflies, but little conclusive information was obtained as to the definite attractant.

For this study large numbers of these blowflies (*Calliphora erythrocephala* Meig., *Lucilia sericata* Meig., and *Phormia regina* Meig.) of known ages were successfully reared in both summer and winter, without the aid of constant temperature and humidity chambers. The males and females, shortly after emergence, were about equal in number, but as the males died more quickly, the flies tested usually consisted of more females than males.

The olfactometer used was the simplest and most satisfactory one yet devised by the writer for work with blowflies. It consisted essentially of a wooden box having a wire-screen top, and of a set of three bottles, one large and the other two small. The bottles were connected on the left with an electric blower pump and on the right by means of glass tubes with two specially constructed cups that fitted into the bottom of the box. The large bottle and one small bottle were half filled with water, and the other small bottle was half filled with the liquid to be tested as an attractant or repellent. The pump forced humid air and odors from the bottles through the tubes and into the cups, and these were diffused in the box through the perforated covers on the cups. The key to success in this investigation was the ready response of blowflies to humid air currents; therefore, humid air, not bearing odors, from one cup served as a control, while humid air bearing odors from the other cup served as the attractant or repellent. The flies lived in the box usually two or three weeks or until the testing was finished. All the testing was done by diffused light in a dark room.

With the aid of the olfactometer it was first determined that the responses of the males and females did not usually differ widely and that the average responses of the males and females of the same species were practically the same.

When fermenting sugar solutions were tested during a period of 25 days, it was determined that solutions not containing baker's yeast were largely attractive, whereas solutions containing baker's yeast were repellent during nearly all of the 25-day period. Tests with carbon dioxide, alcohol, and acetic acid indicated that these substances in the fermenting mixtures had much to do with the responses obtained.

The responses to milk and three of its constituents—lactic acid, lactose, and casein—indicated that they were always attractive. Fermented casein and baker's yeast were among the best attractants found, but putrid meat and putrid eggs were the most attractive.

In order to determine whether or not blowflies smell with their antennae, three series of experiments were conducted. In the first series the antennae, or parts of antennae, of 283 Calliphora were pulled off so that all of the third or terminal segments, which bear the so-called olfactory hairs, were removed. Nine substances, including fresh and fermented milk and brown-sugar water, five concentrations of alcohol, and seven concentrations of lactic acid, were tested. The general average responses, expressed as percentages, obtained were: Unmutilated flies, 68.4; mutiliated flies, 68.2.

In the second series of experiments the antennae of 300 Phormia were pulled off. For a period of 8 days brown-sugar water, milk, yeast mixture, and casein mixture were tested. Five concentrations of alcohol and seven concentrations of lactic acid were also tested. The general average responses were—unmutilated flies, 66.6, and mutilated flies, 67.4.

In the third series of experiments the antennae of 134 Calliphora were pulled off, and after the mutilated flies had been tested, the stumps of their antennae were covered with glue. The average responses to five substances were as follows: Unmutilated flies, 75.7; mutilated flies, 74.9; and flies with glue on the stumps of their antennae, 74.0.

From the preceding averages it is concluded that the antennae of blowflies do not bear the olfactory organs.

## LITERATURE CITED

(1) EYER, J. R., and RHODES, H.
1931. PRELIMINARY NOTES ON THE CHEMISTRY OF CODLING MOTH BAITS. Jour. Econ. Ent. 24 : 702–711, illus.

(2) FRISCH, K. VON
1921. ÜBER DEN SITZ DES GERUSCHSINNES BEI INSECTEN. Zool. Jahrb., Abt. Allg. Zool. u. Physiol. Tiere 38 : 449–516, illus.

(3) HAUSER, G.
1880. PHYSIOLOGISCHE UND HISTOLOGISCHE UNTERSUCHUNGEN ÜBER DAS GERUCHSORGAN DER INSEKTEN. Ztschr. Wiss. Zool. 34 : 367–403, illus.

(4) MCINDOO, N. E.
1914. THE OLFACTORY SENSE OF THE HONEY BEE. Jour. Expt. Zool. 16: 265–346, illus.

(5) ———
1926. AN INSECT OLFACTOMETER. Jour. Econ. Ent. 19 : 545–571, illus.

(6) MINNICH, D. E.
1924. THE OLFACTORY SENSE OF THE CABBAGE BUTTERFLY, PIERIS RAPAE LINN., AN EXPERIMENTAL STUDY. Jour. Expt. Zool. 39 : 339–356, illus.

(7) RIPLEY, L. B., and HEPBURN, G. A.
1929. A NEW OLFACTOMETER SUCCESSFULLY USED WITH FRUIT-FLIES. Union So. Africa Dept. Agr. Ent. Mem. 6 : 55–74, illus.

# PERFECTING A STAND-DENSITY INDEX FOR EVEN-AGED FORESTS[1]

By L. H. REINEKE

*Associate Silviculturist, California Forest Experiment Station, Forest Service, United States Department of Agriculture*

## INTRODUCTION

An adequate expression for density of stocking in even-aged forests has long been sought by foresters. Comparison of total basal area of the stand with yield-table values of basal area for the same age and site quality has been the usual method of evaluating stand density. Other methods have been proposed, but none has given results good enough to warrant general adoption or displacement of the basal-area method. It is the purpose of this paper to present a stand-density index which does not require a yield table and which is not affected by possible errors in shape of the *total basal area-age* curve. This stand-density index, based on the relationship between number of trees per acre and their average diameter, is premised on the characteristic distribution of tree sizes in even-aged stands.

It is a well-established fact that in any given stand a curve showing the relative (percentile) frequency of occurrence of the various tree sizes (diameters) has a characteristic form, often approximating that of the "normal frequency curve" or "normal curve of error" (*1*, *2*, *3*, *4*, *6*, *7*).[2]

## STATISTICAL BASIS

This frequency-curve form may differ with species; the departures from normal may embody positive or negative skewness (*6*), or a logarithmic form may be assumed (*4*). Within a given species, however, stands of all ages on all sites have essentially the same characteristic frequency-curve form (*4*, *7*, *9*).

The form of the frequency curve may be described by several statistical measures, those used most commonly being the average (or mean) diameter and the standard deviation, with the coefficients of asymmetry and of excess less generally used (*6*).

For any one general form of frequency curve, as applied to diameter distribution in even-aged stands, the percentile frequency curve for a specific stand is described primarily by the average diameter. The standard deviation and coefficients of asymmetry and of excess provide a further description of the curve, but since they are correlated with the average diameter they are of secondary importance. It is thus possible to a certain extent to describe the relative diameter distribution of a stand by average diameter alone.

## COMPARISON OF STAND DENSITIES

The concept of stand description is useful in comparing the density of stocking of various stands. Two stands of the same description

[1] Received for publication July 14, 1932; issued May 1933.

[2] Reference is made by number (italic) to Literature Cited, p. 637.

(same average diameter and, by implied correlation, the same standard deviation, etc.) have the same relative distribution of diameters but may differ as to total number of trees per unit area. Obviously, the stand with the greater number of trees is the better stocked or denser stand of the pair and their relative stand densities are directly proportional to the number of trees on them. The number of trees per acre of one stand may be expressed as a percentage of the number of trees of the other; this percentage will indicate the relative stand densities. If data for a sufficient number of stands of the same description (average diameter) are obtained, the stand with most trees per acre can be considered as having 100 per cent stand density. If all other stands are referred to this one, the density of each can be expressed as a percentage of full density.

## THE REFERENCE CURVE

The number of trees per acre for full density varies, however, with the average diameter of the stand. Stands of small average diameter have a large number of trees, while stands of large average diameter have relatively few. To determine the density of stands of all descriptions, it is necessary to have a curve showing the number of trees per acre at full density for all average diameters.

This curve of maximum number of trees per acre over average diameter when plotted on ordinary cross-section paper is concave upwards, falling rapidly in the small diameters and flattening as the larger diameters are reached. When plotted on logarithmic cross-section paper, this curve assumes a straight-line form. For many species the slope of this logarithmic straight-line graph is constant, but its elevation differs with species. This curve is represented by the equation,

$$\log N = -1.605 \log D + k$$

in which $N$ is the number of trees per acre, $D$ is their average diameter (by basal area), and $k$ is a constant varying with species. When $k$ is 4.605 the curve passes through the point representing 10 inches average diameter, 1,000 trees per acre, as shown in Figure 1 (solid line). This curve will be referred to hereafter as the "reference curve."

## CONFORMITY TO THE REFERENCE CURVES

In Figure 2 is shown a series of California red fir (*Abies magnifica* A Murray) yield-plot values as collected by Schumacher (*9*). The reference curve is a very good fit for the maximum values. Similarly, in Figure 3, a curve parallel to the reference curve but passing through 10 inches, 830 trees, expresses the maxima for yield-plot data for white fir (*A. concolor* Lindl. and Gord.).

Other species also conform to the reference curve. Curves parallel to the reference curve are fitted to yield-plot values for the mixed conifer types in California (measured by Dunning, Show, and others) in Figure 4, A, to second-growth Douglas fir (*Pseudotsuga taxifolia* (LaM.) Britt.) in Oregon and Washington (measured by McArdle) in Figure 4, B, and to second-growth Douglas fir in California (measured by Schumacher) in Figure 4, C.

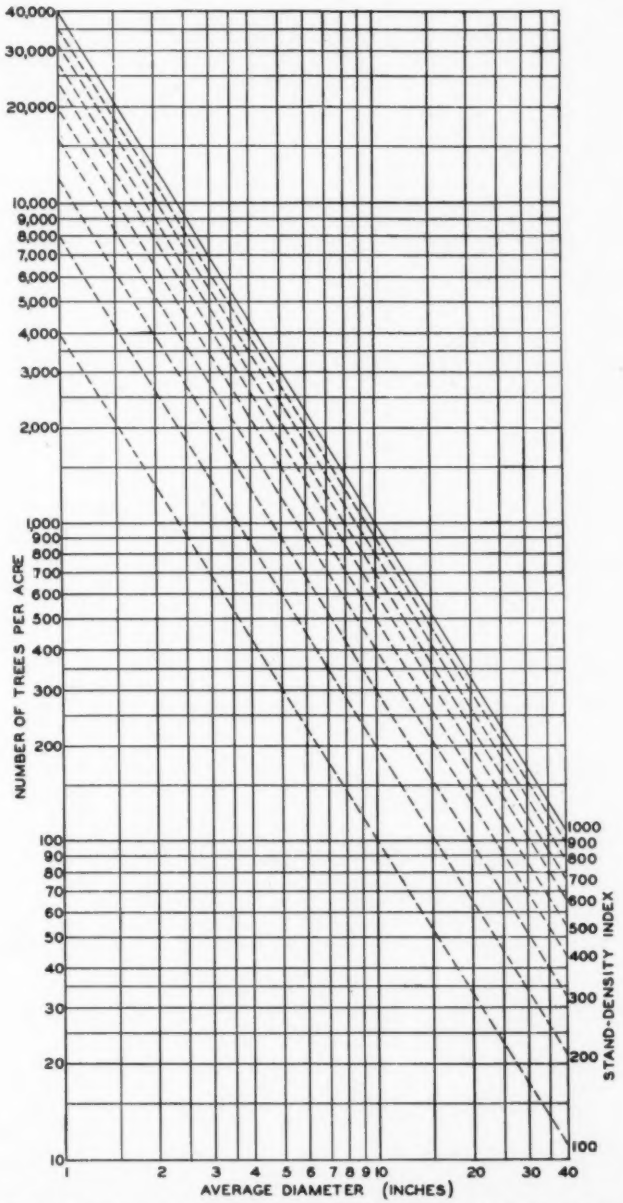


FIGURE 1.—Reference curve (solid line). The stand-density index of each of the broken-line parallel curves is the number of trees indicated by each at 10 inches average diameter

In Figure 5 data are shown for planations of *Eucalyptus globulus* Labill. (*5*), for second-growth ponderosa pine (*Pinus ponderosa* Laws.) as measured by Gallaher, and for second-growth redwood (*Sequoia sempervirens* (Lamb.) Endl.) as measured by D. Bruce. Most of the eucalyptus plantations (fig. 5, A) were widely spaced and

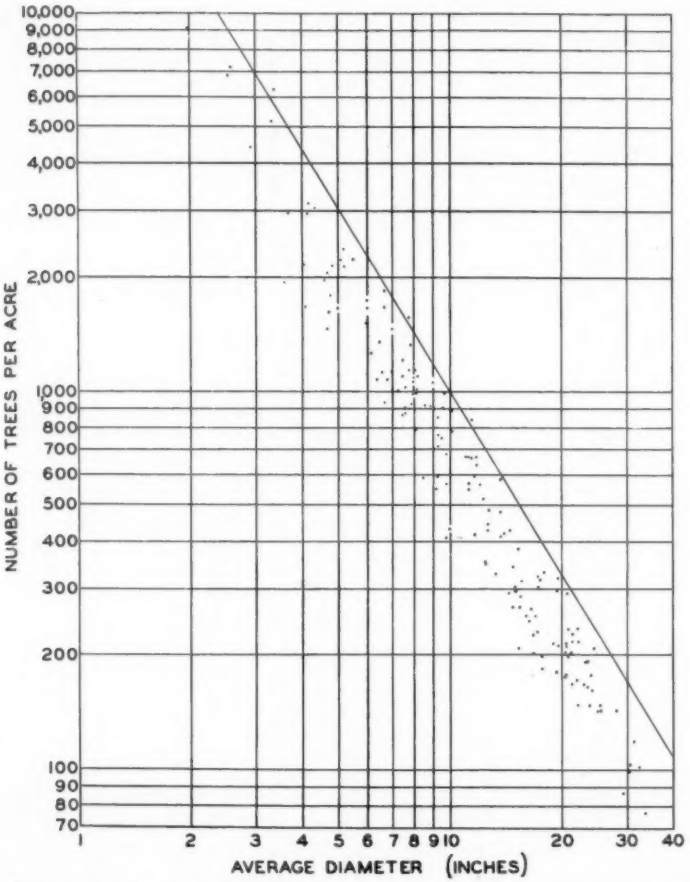


FIGURE 2.—Number of trees—average diameter relation for red fir, with reference curve defining the maxima

very young. They were, therefore, much understocked when measured. The maximum points to which the curve (parallel to the reference curve) has been fitted, represent the few closely spaced or older plantations.

The data for ponderosa pine (fig. 5, C) were taken to represent "overstocked" stands. Although these data cover only a small range in average diameter, they fit the curve of standard slope quite well.

The maximum curve for redwood (fig. 5, B) is less well defined. The distribution of plots by average diameter is poor, however, and additional data below 10 inches and above 20 inches are necessary to establish conformity or nonconformity with the reference curve.

In each of the preceding groups of data, the curve has been fitted to represent the maximum values rather than the average. Ordi-

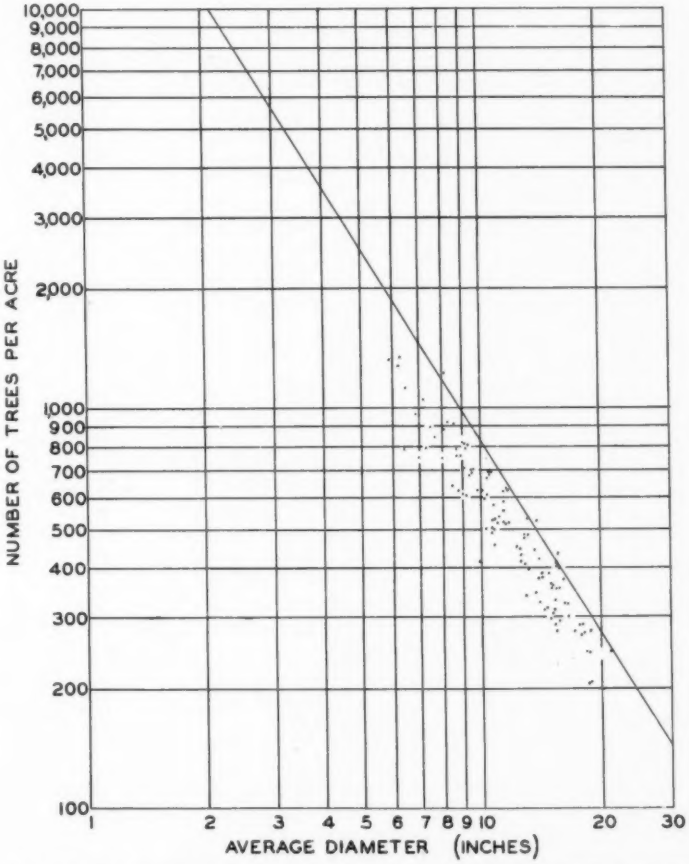


FIGURE 3.—The maximum stand-density index for white fir is 830, as shown by the curve defining the maxima and paralleling the reference curve

narily the average values of any group of data will define a curve better than the maxima, especially if the conditions of random sampling are met. In yield studies, however, biased sampling, as to stand density, is the rule. Plots are selected for maximum stocking and poorly stocked plots are not desired, but in some circumstances normality standards must be lowered to secure adequate representation of stands of certain ages or site quality. This is usually neces-

sary with the larger, older stands; since they are more mature, many of the better stands are cut, leaving only the poorly stocked ones which are less desirable commercially. The result of such a selection would be a lowering of average stand density for large stands, which would lower the large-diameter end of the number of trees—average diameter curve. This distortion is avoided by fitting to the maxima. would lower the large-diameter end of the *number of trees—average diameter* curve. This distortion is avoided by fitting to the maxima.

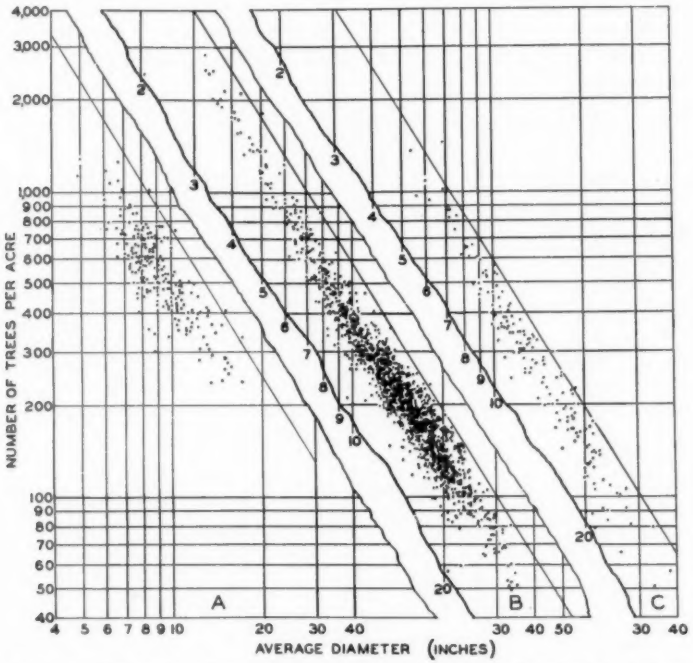


FIGURE 4.—Maxima curves for: A, Mixed conifer stands in California; B, Douglas fir in Washington and Oregon; C, Douglas fir in northern California. Note that the maximum stand-density index is almost identical (approximately 595) for both groups of Douglas fir

Where random sampling is secured, as in strip cruises, fitting to average points is more satisfactory. Figure 6 represents such a case, the data resulting from a strip cruise by G. H. Barnes in second-growth lodgepole pine in British Columbia (*1a*). The numbers in Figure 6 indicate the number of 1-acre plots represented by each average point. The solid line is parallel to the reference curve; the broken (straight) line corresponds to the curve fitted by Barnes on semilogarithmic paper.

In all the above examples, the reference curve, or one parallel thereto, has fitted reasonably well.[3] This is also true for longleaf pine (fig. 7, C) and for loblolly pine (fig. 7, B), based on yield-study plots. In Figure 7, A, showing measurements at 5-year intervals of

[3] This is also true for southern white cedar (*Chamaecyparis thyoides* (L.) B. & P.) and for northern white pine (*Pinus strobus* L.), not illustrated.

two permanent sample plots in loblolly pine, the same slope is indicated. The slope for slash pine, Figure 7, D, is slightly steeper than for the reference curve (broken line), although it is none too well defined. For shortleaf pine, however, the slope is definitely steeper. (Fig. 7, E.)

This possible nonconformity of slash pine, and the definite nonconformity of shortleaf pine, may be due, perhaps, to the influence of fire. Although unburned plots were sought for the yield study, few of them

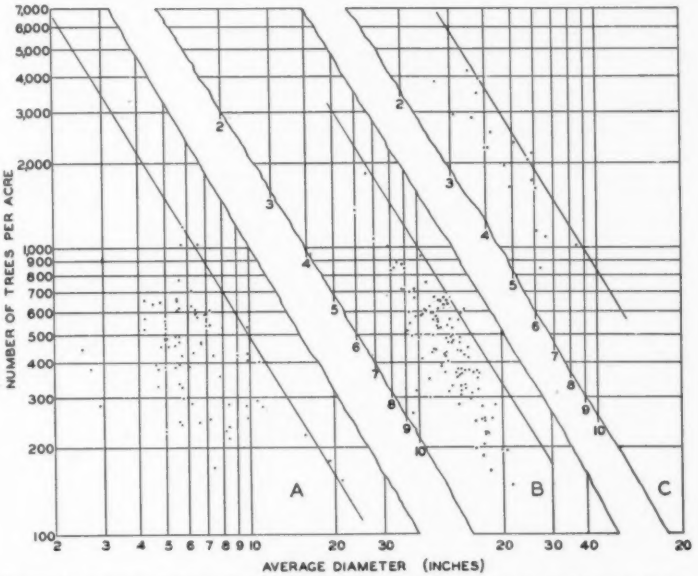


FIGURE 5.—Curves, parallel to the reference curves, for: A, Eucalyptus (in plantations); B, redwood; C, ponderosa pine

were entirely free of fire damage, and the chances of fire injury were obviously greater in the older, larger stands.

## EFFECT OF AGE AND SITE QUALITY

Before accepting this relationship of number of trees per acre to average diameter as an index to stocking, it is necessary to examine into the possible effect of age and site quality. Multiple linear correlations were computed for several species with number of trees per acre in a percentage of the maximum curve as the dependent variable, and with height of average dominant tree of the stand and total age of stand as independent variables. Age and dominant height were used in preference to site index, since the latter would be influenced by any improper shaping of the site-index curves. The regression equations, with the statistical measures, are given in Table 1.

TABLE 1.—*Regression equations and statistical measures resulting from multiple linear correlations of trees per acre, dominant height, and total age of stand*

| Species | Regression equation | Alienation coefficient | Correlation coefficient | Ratio of correlation coefficient to probable error |
|---|---|---|---|---|
| Douglas fir (in California) | Percentage number of trees = −0.067 age +0.011 dominant height+51.075. | 0.990 | 0.141±0.0501 | 2.81 |
| White fir | Percentage number trees=0.006 age+ 0.095 dominant height+56.491. | .978 | .208± .071 | 2.93 |
| Red fir | Percentage number trees=0.229 age− 0.194 dominant height+61.98. | .968 | .072± .049 | 1.47 |

For Douglas fir and white fir, the regression coefficients are very small. For red fir they are considerably larger, but the correlation coefficient is the smallest (0.072) and least significant (correlation coefficient only 1.47 times its probable error). For each species the correlation coefficient is small and insignificant, since it does not exceed three times its probable error.

Apparently, there is no significant or appreciable correlation between age or site quality and the number of trees per acre for a given average diameter. It is, then, safe to use the *number of trees—average diameter* curve as a standard to which similar values of an individual stand may be compared to determine its density of stocking.

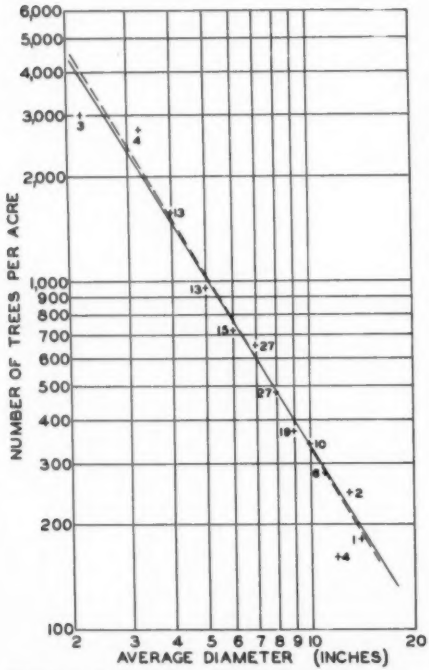


FIGURE 6.—Curve (solid line), parallel to reference curve, fitted satisfactorily to points based on strip cruises in lodgepole pine stands in British Columbia; the broken line corresponds to the curve fitted by Barnes on semilogarithmic paper

## PERCENTAGE STOCKING

The numerical expression of density of stocking may take two forms. The obvious way to express this measure is to establish the position of the *number of trees—average diameter* curve for maximum or 100 per cent stocking for a given species and to express individual stand values as a percentage of this maximum curve. The value thus derived may be termed the "percentage stocking." This is a simple, usable measure, but has the disadvantage of requiring prior determination of the maximum curve. Comparison of one species with another is less simple, because of differences in maxima.

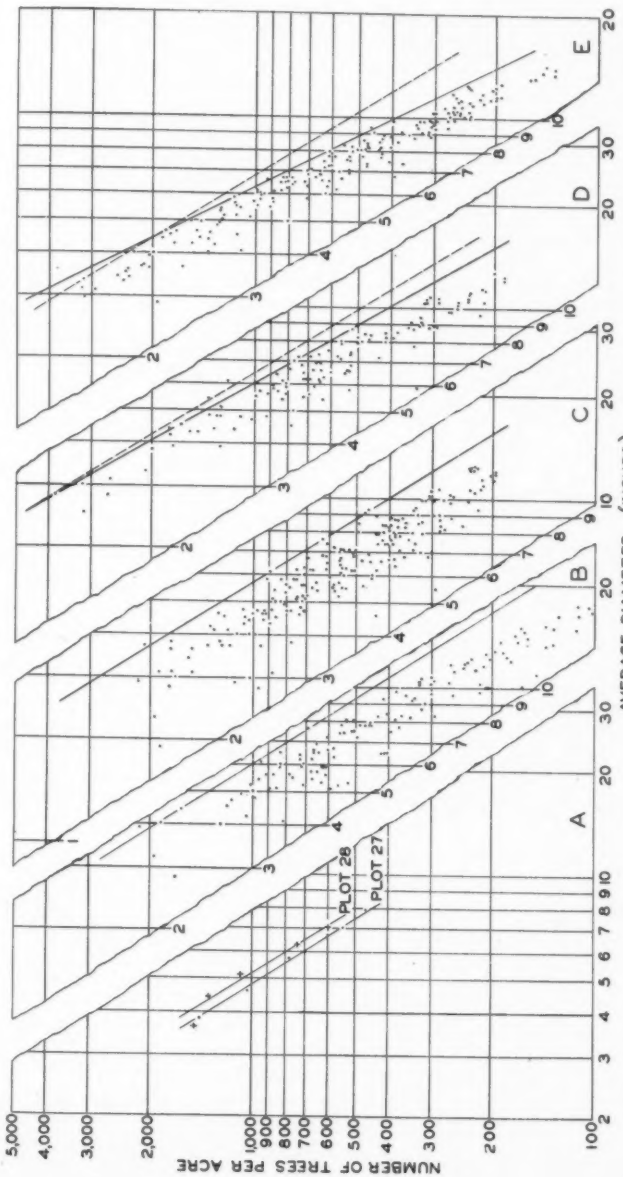


FIGURE 7.—Agreements with and divergences from the reference curve in different species. Parallelism is found in (A) loblolly pine at 5-year intervals, on permanent plots, (B) temporary yield-plot data for loblolly pine, and (C) longleaf pine; divergence is noted to a slight degree in (D, dotted line) slash pine, and more definitely in (E, dotted line) shortleaf pine

## STAND-DENSITY INDEX

A second expression for density, which may be termed the "stand-density index," permits direct comparison between species in addition to expressing varying stand densities within a species. Using the reference curve as the basis for all species for which the slope is the same as that of the reference curve,[4] the number of trees per acre in a given stand is plotted on a graph similar to Figure 1 (with the broken-line curves omitted) and a line is drawn through this point, parallel to the reference curve, until it intersects the 10.0 inch ordinate. The number of trees at this point of intersection is taken as the stand-density index.[5] Since the reference curve passes through 1,000 trees per acre at 10.0 inches, this value is ten times the percentage relation between the stand and the reference curve.

In practice, determination of the 10-inch intercept value by means of the parallel line as described above is not necessary. To Figure 1 has been added a series of broken-line curves parallel to the reference curve and intersecting the 10-inch ordinate at 100, 200, 300 trees, etc., and so labeled. When the average diameter and number of trees per acre of a stand are plotted on this graph, the stand-density index (to the nearest 50 trees per acre) is given by the parallel line nearest the plotted point. By interpolation between curves, stand-density index may be read easily to the nearest 10 trees per acre.

Stand-density index can also be expressed as a percentage of the reference curve. It is deemed more desirable, however, to use the number of trees as the index. This is a quantitative, not a relative, measure and permits a better visualization of stand conditions. Furthermore, the use of number of trees as the index avoids confusion with the percentage stocking values discussed previously.

It is deemed desirable, therefore, to limit the use of percentages to percentage stocking values, for comparison of stands of the same species. For interspecies comparisons, the stand-density index expressed in number of trees is desirable, not only because it is a quantitative measure, permitting better visualization of stand conditions, but because it is directly proportional to the percentage relationship (ten times the latter), thus incorporating any advantages of the percentile values, yet avoiding confusion with percentage stocking.

## CONCLUSIONS

The method of determining density of stocking in even-aged stands, which has been described, has the advantages of simplicity, freedom from correlation with age and site index, and general applicability. The equation derived, expressing the relationship between number of trees per acre and average diameter, satisfies the data for 12 of the 14 species investigated and departs but slightly for the thirteenth. Additional species should be investigaged for conformity with results presented here. For species, or groups of species, not conforming to this equation, separate reference curves may be established, but it is recommended that the number of trees at an average

[4] For species in which the slope differs from that of the reference curve, a curve paralleling its maximum curve and passing through 1,000 trees at 10.0 inches can be used as a reference curve.

[5] This stand-density index is determined very much as is site index, since it is the number of trees per acre with an average diameter of 10.0 inches which the stand had or will have, assuming that the change in number of trees and average diameter progresses parallel with the reference curve.

diameter of 10 inches be maintained as the basis for stand-density index.

Similar investigations of selection stands will be of value. Schæffer (*9*) points out that the distribution of trees by diameter in selection stands can also be represented by a straight line on logarithmic graph paper.

## SUMMARY

The correlation between average diameter of an even-aged stand and the other statistical measures by which the diameter distribution is described permits the use of average diameter alone as a basis for comparing the densities of stands. Of a group of stands of the same average diameter, and, therefore, having the same distribution of diameters, the stand with the greatest number of trees per unit area is obviously the most completely stocked. Age and site quality have no significant effect upon this relationship.

For a given species, the maximum number of trees that it is possible for a stand to have is correlated negatively with the average diameter. The curve representing this relationship assumes a straight-line form when plotted on logarithmic paper and is termed the reference curve.

In 15 groups of data representing 14 species (13 coniferous), the slope of the *number of trees—average diameter* curve was identical for 12 species. The slope for slash pine was slightly greater; that for shortleaf pine was appreciably greater. The heights of these curves, at a given diameter, varied between species.

By means of the reference curve "stand-density index" is derived for a given stand by plotting its number of trees and diameter, passing a line, parallel to the reference curve, through this point, and reading the number of trees per acre at its intersection with the 10-inch ordinate. This expression may be used for comparisons between species and within a species.

Percentage stocking is the percentage expression of the ratio between the number of trees per acre in a given stand and the number, for the same diameter, taken from the maximum curve for the species involved. This expression should be used only for comparisons within a species.

## LITERATURE CITED

(1) Baker, F. S.
1923. NOTES ON THE COMPOSITION OF EVEN-AGED STANDS. Jour. Forestry 21:712–717, illus.

(1a) Barnes, G. H.
1931. THE IMPORTANCE OF AVERAGE STAND DIAMETER AS A FACTOR IN FORECASTING TIMBER YIELDS. 24 p., illus. Brit. Columbia Dept. of Lands, Forest Serv.

(2) Behre, C. E.
1928. PRELIMINARY NORMAL YIELD TABLES FOR SECOND-GROWTH WESTERN YELLOW PINE IN NORTHERN IDAHO AND ADJACENT AREAS. Jour. Agr. Research 37:379–397, illus.

(3) Bruce, D.
1926. A METHOD OF PREPARING TIMBER-YIELD TABLES. Jour. Agr. Research 32:543–557, illus.

(4) ——— and Reineke, L. H.
1929. THE USE OF ALINEMENT CHARTS IN CONSTRUCTING FOREST STAND TABLES. Jour. Agr. Research 38:289–308, illus.

(5) Metcalf, W.
1924. GROWTH OF EUCALYPTUS IN CALIFORNIA PLANTATIONS. Calif. Agr. Expt. Sta. Bul. 380, 61 p., illus.

(6) MEYER, W. H.
1930. DIAMETER DISTRIBUTION SERIES IN EVENAGED FOREST STANDS. Yale Univ. School Forestry Bul. 28, 105 p., illus.
(7) REINEKE, L. H.
1927. A MODIFICATION OF BRUCE'S METHOD OF PREPARING TIMBER-YIELD TABLES. Jour. Agr. Research 35:843–856, illus.
(8) SCHÆFFER, L.
1929. PAPIER LOGARITHMIQUE. Bul. Trimest. Soc. Forest. France-Comté et Provs. Est. 18:[32]–38, illus.
(9) SCHUMACHER, F. X.
1928. YIELD, STAND, AND VOLUME TABLES FOR RED FIR IN CALIFORNIA Calif. Agr. Expt. Sta. Bul. 456, 29 p., illus.

# YELLOWS-RESISTANT LINES OF JERSEY WAKEFIELD CABBAGE [1]

By J. C. WALKER [2]

*Agent, Division of Horticultural Crops and Diseases, Bureau of Plant Industry, United States Department of Agriculture,* and *Professor of Plant Pathology, University of Wisconsin*

## INTRODUCTION

In a previous paper [3] the writer has presented the results of a study of the inheritance of resistance to the yellows organism (*Fusarium conglutinans* Wr.) in cultivated and wild cabbage (*Brassica oleracea* L.). In this earlier paper the symptoms of the disease were described, and data were reported on progenies from resistant and susceptible plants of the varieties All Head Early, Copenhagen Market, and Glory of Enkhuizen and from crosses between resistant and susceptible individuals of these three varieties. From the results of this work it was concluded that resistance is controlled by a single dominant gene (*R*) allelomorphic with a single susceptible gene (*r*). It was also pointed out that all plants belonging to the recessive susceptible class (*rr*) did not, under field conditions, become equally affected by the disease. Some died promptly; others, although diseased, continued to live for varying periods and sometimes recovered. It still remains to be determined whether the expression of the disease may be influenced by other factors than the main gene for resistance.

In the course of the investigation referred to, attention was given to the inheritance of resistance in the Jersey Wakefield variety. Resistant individuals of this variety were self-pollinated. The resulting progenies, when tested upon yellows-infested soil, segregated very nearly in the ratio of 3 resistant to 1 susceptible. Moreover, progenies from selfed individuals showed that the resistant class consisted of homozygous plants (*RR*) and heterozygous plants (*Rr*) approximately in the expected ratio of 1 to 2.

The present paper is a report of a continuation of the study of these lines. The investigation had two main objects: (1) To confirm previous findings regarding the homozygous condition for resistance by testing selected lines through succeeding generations and (2) to determine whether it was possible to combine this resistance with the essential features of the Jersey Wakefield variety. The latter problem is important from the practical standpoint, since it has not heretofore been determined whether resistance in cabbage is linked with certain characters that might involve alteration in the standard

[1] Received for publication Aug. 16, 1932; issued May, 1933. Cooperative investigations between the Department of Plant Pathology, University of Wisconsin, and the Division of Horticultural Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture. Supported in part by a grant from the University Research Fund.

[2] The writer acknowledges with thanks the assistance of F. L. Wellman and L. M. Blank, both of the Division of Horticultural Crops and Diseases, and of M. E. Anderson, formerly of that division, in the execution of field work connected with this problem.

[3] WALKER, J. C. INHERITANCE OF FUSARIUM RESISTANCE IN CABBAGE. Jour. Agr. Research 40: 721–745, illus. 1930.

type of a variety. It has, however, been found by past experience that resistant varieties of cabbage are usually somewhat removed in type from the variety from which they were selected.

## METHODS OF EXPERIMENTATION

The methods of seed growing and field testing for resistance previously described[4] were employed in the present investigation. No attempt, however, will be made to classify the various grades of susceptible plants, since the study of this phase of the general problem is reserved for special treatment. All plants showing signs of yellows were permanently marked for the season and were classed as susceptible, and although the degree of infection was recorded it will not be discussed in this paper.

## EXPERIMENTAL RESULTS

As uniform a stock of commercial Jersey Wakefield as could be secured was planted in 1925 on the yellows-infested field plot in Kenosha County, Wis., hereafter referred to as the yellows plot. The plants were distributed among five lots at different locations in the plot. The total number of plants and the percentage that became diseased in each lot are given in Table 1. It will be seen that the amount of disease varied somewhat but that the resistant plants approximated 20 per cent of the total. In all there were 58 plants that remained free from symptoms of disease throughout the season. Some of these were obviously below the standard for the variety and were discarded. About 35 plants were saved and planted in the greenhouse for seed production during the winter of 1925–26.

TABLE 1.—*Yellows in commercial Jersey Wakefield on the field trial plot in 1925*

| Lot No. | Total plants | Diseased plants | |
|---|---|---|---|
| | *Number* | *Number* | *Per cent* |
| 1 | 67 | 59 | 88.1 |
| 2 | 67 | 58 | 86.6 |
| 3 | 57 | 44 | 77.2 |
| 4 | 67 | 54 | 80.6 |
| 5 | 73 | 58 | 79.5 |
| Total | 331 | 273 | 82.5 |

### FIRST-GENERATION SELECTIONS

Owing to losses from decay of the main stems of the plants during the winter, seed was secured from only 14 plants of the group selected from the resistant survivors of Jersey Wakefield. Seed from self-pollination was obtained from 13 plants, and four sib crosses were made. The behavior of the progenies when tested on the yellows plot in 1926 has already been described.[4] Certain of the progenies were retested in 1927, and the results confirmed those previously obtained. It may suffice, therefore, to point out here that segregation was generally that of a monohybrid, and that when all plants showing any sign of disease were placed in the susceptible class this group contained approximately one-fourth of the individuals.

[4] WALKER, J. C. Op. cit.

Whether the progenies selected for resistance to yellows maintained the characteristic appearance of the type was not considered in the earlier paper. At the time of the initial selections only those plants were retained that conformed reasonably closely to the desired type of Jersey Wakefield. It should be remembered, however, that cabbage is an open-pollinated plant and that the individual may therefore be expected to be heterozygous for many of its characters.

Table 2 shows the data on the first-generation progenies. It will be seen that all the progenies segregated for resistance and susceptibility and that approximately 25 per cent of the plants were in the susceptible class. Data on type were taken for the plants in the resistant class, which contained approximately 75 per cent of the total. Since only the outstanding points are considered here, the characteristics ascribed to a given progeny apply to most but not necessarily to all individuals in that progeny. Although this study was not concerned with the inheritance of any character of cabbage other than resistance, in the selection of resistant strains an effort was made to maintain or improve the type.

TABLE 2.—*Results of field trials on yellows-infested soil with progenies of first selections from Jersey Wakefield, as compared with two commercial susceptible varieties*

PROGENIES FROM SELFED PLANTS AND SIB CROSSES OF JERSEY WAKEFIELD

| Parent plant No. | Year of trial | Total plants tested | Plants showing yellows | Notes on resistant plants |
|---|---|---|---|---|
| | | *Number* | *Per cent* | |
| 3 | 1926 | 25 | 16.0 | Late maturity; stems too long. |
| 6 | 1926 | 117 | 31.6 | Late. |
| 7 | 1926 | 71 | 25.4 | Early; good type. |
| | 1927 | 22 | 27.3 | Do. |
| 8 | 1926 | 32 | 31.3 | Early; heads too slender at base. |
| 9 | 1926 | 85 | 27.1 | Late; heads too slender at base. |
| 10 | 1926 | 185 | 14.6 | Early; good type. |
| | 1927 | 92 | 23.9 | Do. |
| 12 | 1926 | 216 | 24.1 | Late; coarse; irregular. |
| 15 | 1926 | 94 | 9.6 | Fairly early; good type. |
| 17 | 1926 | 30 | 13.3 | Late. |
| 19 | 1926 | 17 | 29.4 | Do. |
| 21 | 1926 | 180 | 23.3 | Early; good type. |
| | 1927 | 173 | 28.3 | Do. |
| 29 | 1926 | 61 | 19.7 | Late; coarse; leafy. |
| 34 | 1926 | 139 | 21.6 | Late; stems too long. |
| 6×3 | 1926 | 36 | 19.4 | Poor showing of selfs of two parents. |
| 7×10 | 1926 | 177 | 29.4 | Early; excellent type. |
| | 1927 | 180 | 34.4 | Do. |
| 11×12 | 1926 | 61 | 32.8 | Early; good type. |
| 21×15 | 1926 | 121 | 25.6 | Early; excellent type. |
| | 1927 | 31 | 19.4 | Do. |
| Total | | 2,145 | 24.6 | |

COMMERCIAL SUSCEPTIBLE VARIETIES

| Variety | Year of trial | Total plants tested | Plants showing yellows |
|---|---|---|---|
| | | *Number* | *Per cent* |
| Danish Ballhead | 1926 | 41 | 82.9 |
| | 1927 | 251 | 96.4 |
| Copenhagen Market | 1926 | 240 | 87.5 |
| | 1927 | 255 | 80.0 |

It will be seen that the average date on which many of the progenies matured was later than desirable. Of the strains that were satisfactory for earliness, all but one, that from plant 8, approached the ideal for other type characters. Selections were again made from the best progenies including selfed progenies from plants 7, 10, 15, and 21 and from sib crosses 7×10, 11×12, and 21×15.

### SECOND-GENERATION SELECTIONS

From the plants selected from the first-generation progenies, seed was secured in the greenhouse in the winter of 1926–27 by self-pollination, by brother-sister matings, and by crossing with plants of a

FIGURE 1.—Trial plot on which cabbage progenies were tested for resistance to the yellows organism. Bamboo stakes mark diseased plants: A, Self progeny from plant 155, which is segregating into resistant and susceptible classes; B, hybrid progeny 116×137 from a cross between homozygous resistant plant 116 and plant 137, which was heterozygous for this character; since the resistant gene is dominant, all plants remained healthy; C, self-progeny from homozygous resistant plant 116; all plants remained healthy; D, susceptible Copenhagen Market variety almost completely killed by the disease. (For further data, see Table 3)

homozygous susceptible line. The breeding behavior of these plants in regard to resistance and susceptibility has been discussed in the earlier paper.[5] It was shown that of the resistant survivors selected from the first-generation progenies approximately one-third were homozygous for resistance (*RR*) and the remainder were heterozygous (*Rr*). The breeding behavior as to type of the progenies from self-pollination and sib crossing is summarized in Table 3. The appearance of certain of the progenies in the field is illustrated in Figure 1.

[5] WALKER, J. C. Op. cit.

TABLE 3.—*Results of field trials on yellows-infested soil with second-generation progenies from Jersey Wakefield, as compared with three commercial susceptible varieties*

PROGENIES FROM SELFED PLANTS AND SIB CROSSES OF JERSEY WAKEFIELD

| Parent plant No. | Genotype class of parent plant | Year of trial | Total plants tested | Plants showing yellows | Notes on resistant plants |
|---|---|---|---|---|---|
| | | | *Number* | *Per cent* | |
| 116 | *RR* | 1927 | 30 | 0 | Shows loss of vigor; late. |
| 117 | *RR* | 1927 | 34 | 0 | Early; off-color. |
| 124 | *RR* | 1927 | 20 | 0 | Show loss of vigor; late. |
| 135 | *RR* | 1927 | 7 | 0 | Shows loss of vigor. |
| 140 | *RR* | 1927 | 41 | 0 | Early; uniform; good type. |
| | | 1928 | 20 | 0 | Do. |
| | | 1929 | 35 | 0 | Do. |
| 154 | *RR* | 1927 | 74 | 0 | Do. |
| | | 1928 | 17 | 0 | Do. |
| | | 1929 | 25 | 0 | Do. |
| 115 | *Rr* | 1927 | 30 | 26.7 | Late; off-color. |
| 137 | *Rr* | 1927 | 26 | 23.1 | Shows loss of vigor; late. |
| 138 | *Rr* | 1927 | 13 | 46.2 | Shows loss of vigor. |
| 155 | *Rr* | 1927 | 32 | 34.4 | Do. |
| 157 | *Rr* | 1927 | 59 | 28.8 | Uneven in maturity. |
| 158 | *Rr* | 1927 | 34 | 26.5 | Shows loss of vigor. |
| 124 × 135 | *RR* × *RR* | 1927 | 19 | 0 | Early; good type. |
| 140 × 116 | *RR* × *RR* | 1927 | 36 | 0 | Do. |
| | | 1928 | 30 | 0 | Do. |
| | | 1929 | 53 | 0 | Do. |
| 115 × 135 | *Rr* × *RR* | 1927 | 45 | 0 | Early. |
| 116 × 137 | *RR* × *Rr* | 1927 | 45 | 0 | Late. |
| 117 × 139 | *RR* × *Rr* | 1927 | 30 | 0 | Early. |
| 135 × 115 | *RR* × *Rr* | 1927 | 9 | 0 | |
| 154 × 136 | *RR* × *Rr* | 1927 | 17 | 0 | Do. |
| 158 × 124 | *Rr* × *RR* | 1927 | 24 | 0 | Late. |
| 157 × 117 | *Rr* × *RR* | 1927 | 73 | 0 | Early. |
| 131 × 109 | *Rr* × *Rr* | 1927 | 27 | 29.6 | Late; heads poorly shaped. |
| 138 × 155 | *Rr* × *Rr* | 1927 | 32 | 25.0 | Late. |

COMMERCIAL SUSCEPTIBLE VARIETIES

| Variety | Year of trial | Total plants tested | Plants showing yellows |
|---|---|---|---|
| | | *Number* | *Per cent* |
| Danish Ballhead | 1927 | 251 | 96.4 |
| | 1928 | 298 | 94.6 |
| | 1929 | 293 | 91.1 |
| Jersey Wakefield | 1928 | 152 | 90.8 |
| Copenhagen Market | 1927 | 255 | 80.0 |
| | 1928 | 61 | 90.2 |

It will be seen that further segregation as to type occurred. Many progenies from self-pollinated plants showed a decided reduction in vigor, as the writer has previously observed in cabbage. Two progenies, however, were outstanding in holding a remarkable degree of uniformity of earliness and other desirable characters along with vigor. These were progenies from the self-pollination of plants 140 and 154. Of the progenies from sib crosses, 124×135 and 140×116 were equally satisfactory, although of the four parent plants entering into these crosses only plant 140 produced a superior progeny when self-pollinated.

Since the four progenies just mentioned were all homozygous for resistance they became the source of plants for further selection. All other lots were discarded.

SUBSEQUENT GENERATIONS

Since a fairly high degree of uniformity in type and maturity had been attained in the progenies saved from the second generation, no further self-pollination was practiced, except in one case noted later. Groups of plants were planted in isolated places, and cross-pollination by means of insects was allowed to follow. The seeds from plants in each such lot were mixed. The sources of the mother seed plants and the lot numbers of the seed secured were as follows:

| Seed lot No. | Source of mother seed plants |
|---|---|
| 20–28–A | Self progeny of plant 140. |
| 20–201s[6] | Progeny 124 × 135. |
| 20–29–C | Progeny 140 × 116. |
| 20–30–A | Seed lots 20–28–A and 20–29–C. |
| 20–30–B | Progenies 140 × 116, 201s, and seed lot 20–29–C. |

FIGURE 2.—Sample individuals taken from homozygous resistant lines of Jersey Wakefield cabbage, at maturity. These are reasonably close to the types represented in acceptable commercial susceptible stocks of this variety. The conical-shaped head is characteristic. Note that the outer leaves of the head extend to the apex or overlap it slightly. A, Head taken from seed lot 20–29–C. B, Plant from lot 20–201s

All the seed lots listed were tested on yellows-infested soil either in the yellows plot or in the greenhouse. The results of these trials (Table 4) show that the plants were as completely resistant as the progenies from which they were derived. It may be concluded therefore, that resistance was completely fixed in these lines. Sample individuals are illustrated in Figure 2.

TABLE 4.—*Results of tests on yellows-infested soil of strains derived from homozygous resistant second-generation progenies*

| Seed lot No. | Where tested | Plants tested | Plants diseased |
|---|---|---|---|
| | | *Number* | *Number* |
| 20–28–A | Field | 280 | None. |
| 20–201s | do | 170 | Do. |
| 20–29–C | do | 365 | Do. |
| 20–30–A | Greenhouse | 94 | Do. |
| 20–30–B | do | 72 | Do. |
| | Field | 300 | Do. |

[6] In this lot only one plant blossomed. The blooming plant was selfed.

## COMPARISON OF THE RESISTANT STRAINS WITH COMMERCIAL JERSEY WAKEFIELD

It has been pointed out that during the course of this work a special effort was made to select for uniformity of maturity and for the desirable characters of the Jersey Wakefield variety. After seed had been secured in sufficient quantity, comparative tests were made in 1931 at Madison, Wis., of three of the lots listed on page 644, together with four lots of Jersey Wakefield secured from commercial sources. Each lot was planted in double rows, 30 plants to a row, on soil free from yellows, and the complete series was run in triplicate. Seed was sown in flats in the greenhouse on April 1. Plants were pricked out into flats and placed in a coldframe on April 17 and set into the field on May 22. The season was decidedly favorable for cabbage development, except that at the time when heading began the air temperature was unusually high. This appeared to increase somewhat the degree of irregularity in maturity.

The first mature heads were noted on the fifty-third day after transplanting. The number of mature marketable heads was recorded at 3-day to 4-day intervals thereafter. Table 5 shows the percentage of mature plants in each lot on the fifty-third day and at regular intervals up to the seventy-fifth day. It will be seen that considerable variation occurred among the four commercial lots. Lot 4 had the largest number of mature heads on the fifty-third day, and this lot continued to mature more rapidly than the others. Lot 2 was decidedly the slowest of the commercial lots in maturing.

TABLE 5.—*Number of days from transplanting to maturity for various commercial and resistant strains of Jersey Wakefield at Madison, Wis., 1931*

| Period from transplanting to maturity (days) | Percentage of plants reaching mature-head stage in— | | | | | | |
|---|---|---|---|---|---|---|---|
| | Commercial strain No. | | | | Resistant strain No. | | |
| | 1 | 2 | 3 | 4 | 20–28–A | 20–30–A | 20–30–B |
| 53 | 16 | 8 | 13 | 24 | 37 | 7 | 8 |
| 56 | 29 | 13 | 23 | 33 | 63 | 17 | 23 |
| 59 | 47 | 23 | 38 | 46 | 73 | 25 | 34 |
| 62 | 55 | 28 | 50 | 59 | 81 | 36 | 51 |
| 65 | 78 | 58 | 67 | 80 | 90 | 56 | 76 |
| 68 | 86 | 73 | 80 | 90 | 92 | 68 | 86 |
| 72 | 92 | 79 | 86 | 92 | 95 | 72 | 91 |
| 75 | 93 | 85 | 90 | 94 | 97 | 80 | 94 |

Resistant strain 20–28–A was distinctly superior to all other lots not only in the number mature on the fifty-third day but also in the promptness with which the remaining plants came to head. It is to be recalled that this lot was derived from the selfed progeny of plant 140. Each of the two other resistant strains was derived from plants from several progenies. They did not mature as promptly as 20–28–A. 20–30–B was better in this respect than 20–30–A. Both were in the range represented in the commercial lots. These data show that so far as time of maturity is concerned the resistant strains are within the limits represented by the commercial variety from which they were originally derived.

For comparing the relative earliness of the various susceptible and resistant lots, the most satisfactory criterion is the time required for 50 per cent of the plants in a given lot to reach maturity. On this basis 20–28–A was distinctly superior to all other lots, for 63 per cent of the plants of this lot were mature 56 days after transplanting. No other lot reached this stage until the sixty-second day, when susceptible lots 1, 3, and 4 and resistant lot 20–30–B showed 50 per cent or more mature plants. Susceptible lot 2 and resistant lot 20–30–A did not reach this stage of development until the sixty-fifth day.

To secure a comparison of other characters, certain measurements were made of the first 50 heads that matured in each lot. These measurements are given in Table 6. The three resistant strains were uniformly about 2 cm less in height of plant than the susceptible lots. Susceptible lot 2 had the greatest spread of plant; all other lots were reasonably similar in this respect. In number of outer leaves 20–28–A was lowest, and susceptible lot 2 was highest. The other five lots showed little variation in this character.

In weight of outer leaves there was considerable variation. This is an important character and should be considered in relation to weight of head. Seasonal conditions, of course, influence greatly the proportion of outer leaf to head. In 1931 at Madison the proportion of weight of leaf to weight of head was unusually high, probably because of the extremely high temperatures when the plants were approaching maturity. It will thus be seen in the calculated ratios of leaf weight to head weight that the latter ranged from 50 to 91 per cent of the former. Under most favorable seasonal conditions it might have averaged 150 to 200 per cent. Under the conditions of this experiment susceptible lot 2 showed the largest amount of leaf weight in proportion to head weight. Resistant strain 20–30–A was also relatively high in leaf weight. Lots 3 and 4 were the lowest of the susceptible strains in relative leafiness, and resistant strain 20–30–B was very close to them. Of all the strains tested, however, 20–28–A had the lowest leaf weight in proportion to head weight.

The various measurements of length and diameter of stem show no important differences among the several strains. The length of head from top to base and the diameter (taken at the point of greatest diameter) were considered in relation to each other. The length ranged from 1.39 times the diameter in commercial strain 2 to 1.64 times the diameter in resistant strain 20–30–B. Length of core is also an important feature and may be considered in relation to length of head. Relative shortness of core is, of course, desirable. Ratio of length of core to length of head ranged from 1:2.08 in resistant strain 20–28–A to 1:2.57 in 20–30–B. Resistant strain 20–30–A and all the susceptible lots fell between these extremes.

The characters of outer leaves in the various lots showed no tangible differences except in color and bloom. In the resistant strains there was slightly less bloom than in the susceptible ones. Reduction in bloom tended to give a yellower cast to the foliage as well as to the outer leaves of the head. This deviation, however, was not considered sufficient to detract materially from the commercial value of the resistant strains. Furthermore it is to be noted that the amount of bloom varies with season and soil.

TABLE 6.—*Physical measurements of various commercial and resistant strains of Jersey Wakefield, grown at Madison, Wis., 1931*

| Measurements | | Commercial strain No.— | | | | Resistant strain No.— | | |
|---|---|---|---|---|---|---|---|---|
| | | 1 | 2 | 3 | 4 | 20-28-A | 20-30-A | 20-30-B |
| Height of plant | centimeters | 23.28± 0.34 | 24.5 ± 0.42 | 23.54± 0.42 | 23.10± 0.23 | 21.28± 0.20 | 21.16± 0.32 | 20.98± 0.25 |
| Spread of plant | do | 48.76± .51 | 50.90± .67 | 46.56± .55 | 48.0 ± .64 | 46.06± .45 | 47.1 ± .75 | 46.8 ± .46 |
| Outer leaves | number | 13.8 ± .37 | 15.14± .32 | 13.5 ± .38 | 13.9 ± .32 | 12.96± .34 | 14.6 ± .32 | 14.0 ± .23 |
| Weight of outer leaves | grams | 398.5 ±12.37 | 534.9 ±21.40 | 363.1 ±11.85 | 413.8 ±10.18 | 359.1 ± 8.03 | 402.0 ±13.25 | 410.5 ± 8.49 |
| Length of stem: | | | | | | | | |
| From soil to base of first leaf | centimeters | 4.54± .20 | 3.87± .16 | 3.17± .13 | 3.57± .16 | 2.95± .10 | 3.31± .18 | 2.68± .12 |
| From base of first leaf to base of head | do | 5.89± .13 | 5.10± .11 | 5.32± .10 | 5.52± .14 | 5.34± .10 | 5.59± .11 | 5.76± .10 |
| Diameter of stem: | | | | | | | | |
| At soil line | do | 1.46± .02 | 1.68± .02 | 1.36± .02 | 1.54± .02 | 1.46± .02 | 1.38± .02 | 1.36± .03 |
| At first leaf | do | 2.22± .04 | 2.91± .06 | 2.45± .04 | 2.35± .03 | 2.56± .03 | 2.50± .04 | 2.46± .03 |
| At base of head | do | 3.08± .04 | 3.63± .04 | 3.45± .03 | 3.38± .03 | 3.55± .03 | 3.57± .03 | 3.65± .09 |
| Weight of head | grams | 259.4 ± 8.41 | 304.2 ±12.57 | 262.6 ± 8.70 | 290.0 ± 9.31 | 329.7 ± 7.70 | 239.4 ± 8.58 | 295.2 ± 9.58 |
| Ratio of weight of outer leaves to weight of head | | 1.0 : .65 | 1.0 : .57 | 1.0 : .72 | 1.0 : .70 | 1.0 : .92 | 1.0 : .60 | 1.0 : .72 |
| Length of head | centimeters | 11.05± .16 | 11.04± .18 | 11.44± .18 | 11.55± .17 | 11.46± .17 | 10.45± .14 | 12.6 ± .15 |
| Diameter of head [a] | do | 7.12± .10 | 7.97± .15 | 7.05± .11 | 7.49± .09 | 8.20± .10 | 7.06± .11 | 7.66± .11 |
| Length of core | do | 4.75± .10 | 4.47± .11 | 4.97± .08 | 5.25± .10 | 5.50± .11 | 4.63± .07 | 4.9 ± .09 |
| Diameter of core [b] | do | 2.56± .02 | 2.77± .03 | 2.60± .03 | 2.79± .03 | 2.84± .03 | 2.72± .03 | 2.79± .03 |
| Ratio of diameter of head to length of head | | 1.0 : 1.55 | 1.0 : 1.39 | 1.0 : 1.62 | 1.0 : 1.54 | 1.0 : 1.40 | 1.0 : 1.48 | 1.0 : 1.64 |
| Ratio of length of core to length of head | | 1.0 : 2.33 | 1.0 : 2.47 | 1.0 : 2.30 | 1.0 : 2.20 | 1.0 : 2.08 | 1.0 : 2.26 | 1.0 : 2.57 |

[a] Taken at point of maximum diameter.

[b] Taken midway between base and apex.

## DISCUSSION

In the course of this report it has been shown that homozygous resistant progenies selected from commercial Jersey Wakefield have been repeatedly secured. Rigid selection for conformity to type within these lines was practiced and three distinct strains were developed and carefully compared for maturity and important physical measurements with four commercial stocks of the original variety. One of these resistant lines was derived directly from a selfed progeny and each of the two other lines from a combination of two or more resistant progenies that were allowed to cross-pollinate. The object in varying the procedure was to determine the feasibility of practicing self-pollination until resistance was fixed and other desirable characters were also more stable and then continuing the line by sib crossing within the progeny. Although some progenies showed distinct loss of vigor from selfing, others showed no detrimental effects. One of the latter (the self progeny from plant 140) was sib crossed and yielded seed lot 20–28–A.

The data in Tables 5 and 6 show that two of the resistant lines, 20–28–A and 20–30–B, were reasonably like the better commercial stocks 1, 3, and 4. Commercial Jersey Wakefield 2 was obviously a poor stock of the variety, since it was later in maturity than is desirable and tended to show excessive leafiness. Of the two resistant strains, 20–28–A was distinctly superior to all strains in earliness and in proportion of leaf weight to head weight. It had, however, two objectionable features, namely, the diameter of the head, which was relatively greater, and the core, which tended to be longer, than those of acceptable commercial strains. Resistant strain 20–30–B, on the other hand, was much closer to the three best susceptible stocks. Although it was not so early as 20–28–A, it compared favorably in this respect with susceptible stocks 1, 3, and 4. Furthermore, it was nearer the accepted commercial strains in head diameter and core length. In view of these characters, it is probably better fitted for increase and general use than is 20–28–A.

As a result of this investigation, the writer wishes to emphasize the desirability of most careful and rigid selection for type in the course of improvement of cabbage for resistance to yellows. In the work presented here it has been possible to combine resistance with the more important type characters found in acceptable commercial stocks, without prolonging the program through repeated back crossing to the susceptible stock. The latter procedure should be used, however, when it becomes necessary to accumulate in the resistant line the genes controlling desirable characters.

## SUMMARY

The evidence presented in this paper confirms the results reported earlier, from which it was concluded that a single-factor difference exists between plants highly resistant or highly susceptible to the yellows organism. It is further demonstrated that from resistant survivors occurring when the Jersey Wakefield cabbage is planted on infested soil homozygous resistant pure lines can be secured which continue to be completely resistant in succeeding generations.

In the course of this investigation it was found that by rigid selection for type and uniformity of maturity it is possible to combine the desirable characters of this variety with resistance.

# THE EFFECTS OF NONBENEFICIAL NODULE BACTERIA ON AUSTRIAN WINTER PEA[1]

By LEWIS T. LEONARD, *Bacteriologist, Soil Investigations, Bureau of Chemistry and Soils*, and W. R. DODSON, *Agent, Animal Husbandry Division, Bureau of Animal Industry, United States Department of Agriculture*[2]

## INTRODUCTION

When typical nodules form on the roots of legumes the bacteria which they contain are presumed to be working in harmony with the plants, furnishing nitrogenous compounds manufactured from the gaseous nitrogen of the air. Apparently this is not always the case, however, for occasionally a condition arises in which there is no doubt that the nodule organisms have exerted a detrimental effect. In many other instances it is probable that the bacteria live on the roots of the legume without appreciably influencing the plant. It is to be expected that all grades of efficiency exist among the legume bacteria.

## REVIEW OF LITERATURE

The nature of the association of legume and nodule bacteria has long been in doubt. By some it has been regarded as parasitism, but if it is so, it can only be considered as theoretical parasitism. Voorhees and Lipman (*12, p. 92*)[3] state:

> * * * in the very definition of the term, parasitism involves a more or less marked injury to the host, which, at best, derives no benefit from the invading organism. In the phenomenon under consideration, however, there is, under normal conditions, a distinct gain to the host plant, and hence the term "parasitism" is not applicable.

Woronine (*13*), who first discovered bacteria in nodules, referred to them as parasites. Smith (*10, v. 2, p. 97*), considered nodule bacteria as parasites, since they pass through the same stages as disease-producing organisms. Jones (*7*) attributes parasitic tendencies to mung-bean nodules growing under submerged conditions.

Eaton (*4*) has reported that nodule production on soybeans bears a direct relation to light, and Leonard (*8*), also working with soybeans, has shown that a lack of light, or interference with the organs utilizing light, reduces the beneficial effects of symbiosis in accordance with the severity of the light deficiency. Thornton (*11*) states that parasitism in nodule bacteria is induced by cutting off the carbohydrate supply.

The appearance of ill effects among legume plants growing under optimum conditions is due to another type of parasitism—a parasitism inherent in the invading organism. In cases of this kind the organism enters the roots and forms nodules, but these are not beneficial.

---

[1] Received for publication June 8, 1932; issued May, 1933.

[2] The services of Daniel Ready, Bureau of Chemistry and Soils, and of A. P. Kerr, Louisiana Agricultural Experiment Station, in making nitrogen and other analyses, and of J. B. Thompson, Bureau of Plant Industry, in assisting in field and laboratory work, are greatly appreciated.

[3] Reference is made by number (italic) to Literature Cited, p. 663.

Hiltner (*6*) calls attention to parasitic nodules on locust and differentiates between these and beneficial nodules by their location on the roots. The former were distributed all over the root, whereas the latter were located at the crown.

Helz, Baldwin, and Fred (*5*) citing 16 papers on this subject, reported a wide variation in the effects produced by strains of nodule bacteria of the vetch and pea group.

Briscoe [4] reported greenhouse work in which poor and good strains were used in combination, and the mixture produced as satisfactory results as the good strain alone. A means of correcting the unadaptability of strains is reported by Wunchik (*14*), who has indicated that by plant passage an unadapted strain may become better adapted to a strange host, and Allen and Baldwin (*1*) have confirmed this finding. That the organisms in nodules on the same plant may consist of both good and poor strains has been shown by Dunham and Baldwin (*3*).

## EXPERIMENTAL MATERIALS AND OUTLINE OF WORK

In 1929 observations were made at Jeanerette, Iberia Parish, La., on a field of Austrian winter peas (*Pisum arvense* L.) which had been planted without artificial inoculation on the low-lying black neutral alluvial soil of that locality. After a month or so of growth it was definitely apparent that the crop would be a failure; the field contained mainly yellow stunted plants with an occasional vigorous individual. Samples of the plants were collected and sent to the senior author at Washington, D. C. The roots of most of the plants examined, whether normal or stunted, were apparently healthy, and all the plants had nodules on their roots. It was noticed, however, that the nodules on the stunted plants were small, globular in shape, and rather evenly distributed over the roots, whereas those on the vigorous plants tended to be convolute or branched and were largely confined to the crown roots. Cultures were isolated from nodules of both types of plants and tested on Austrian winter pea. These cultures produced nodules on the pea under controlled conditions and, in addition, gave rise to effects similar to those noticed on the plants from which they came. In other words, the bacteria from the stunted-plant nodules produced what might be called parasitic effects, whereas those from vigorous plants produced good plants. When these preliminary tests were completed cultures of the proper nodule bacteria were prepared on agar in test tubes and sent to Jeanerette, La. (Table 1.) There they were applied to seed by adding water to the tube, shaking thoroughly to secure a suspension of the organisms, and moistening the seed with the mixture. The seeds were sown promptly after treatment at the rate of 1 pound per each 10 by 60 foot plot. To prevent transfer of the cultures, vessels and hands were thoroughly rinsed in running tap water between each treatment. No disinfectants were used. Before the seeds were sown basic slag at the rate of 600 pounds per acre and ground oyster shells at the rate of 3,600 pounds per acre were applied each to one-third of the plots in Austrian winter pea; the remaining third were left untreated. Figure 1 shows the arrangement of the plots.

[4] BRISCOE, C. F. INOCULATION OF SOYBEANS. Miss. Agr. Expt. Sta. Press Circ. 419, 3 p. 1932. [Mimeographed.]

TABLE 1.—*Source of the nodule bacteria cultures used in the experiments*

| Stock No. | Diagram No. (fig. 1) | Host plant from which bacteria were isolated | Source of cultures |
|---|---|---|---|
| 473[a] | 1 | Alaska garden pea | W. H. Wright, University of Wisconsin; his No. P3. 1927. |
| 484[a] | 7 | do | I. L. Baldwin, University of Wisconsin; his No. 15 or 310 1927. |
| 510 | 3 | Austrian winter pea | Jeanerette, La 1929. |
| 511 | 6 | do | Do. |
| 512 | 4 | do | Do. |
| 513[b] | 2 | do | Do. |
| 515[b] | 8 | do | Do. |
| 518[c] | 5 | do | Rosslyn, Va. 1930. |
| None[d] | [d]0 | | |
| 548 | | Louisiana vetch | Jeanerette, La. 1931. |
| 549 | | do | Do. |
| 549 | | do | Do. |
| 550 | | do | Do. |

[a] The complete history of this culture is not available, but judging from the response obtained with Austrian winter peas, it is believed to be a low nitrogen-fixing strain.
[b] This culture was isolated from plants that showed no benefit from the presence of nodule organisms.
[c] This culture was isolated from a slightly pink nodule apparently produced by a nonbeneficial culture on plants showing symptoms of being adversely affected by nodule bacteria. From subsequent results it is presumed that this culture either changed its character rapidly, by means of one plant passage, or that through some unknown means an efficient organism was harbored in the nodule from which it came.
[d] Designation used for untreated areas.

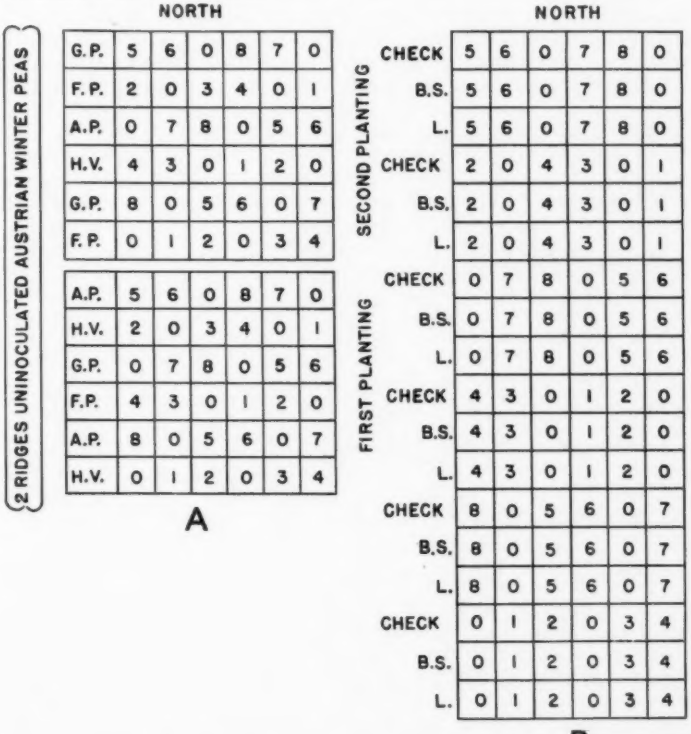


FIGURE 1.—Arrangement of plots of Austrian winter peas and other legumes at Jeanerette, La., 1930: A, Detail of plot arrangement of mixed-legume plantings; B, detail of plot arrangement of Austrian winter-pea plantings. The letters to the left of the numbers indicate the species of legume planted, or a soil amendment, as follows: A. P., Austrian winter pea; F. P., Canada field pea; G. P., garden pea; H. V., hairy vetch; B. S., basic slag; L., ground oyster shell lime

The mixed-legume plots and one of the Austrian winter-pea plots (fig. 1, B) were seeded October 22, 1930; the second sowing of Austrian winter peas, a duplicate of the first, was made November 3, 1930. The plots seeded to mixed legumes were 10 by 60 feet, and those seeded to Austrian winter peas were 10 by 20 feet. Samples of the black silt soil taken from seven representative spots in the mixed-legume planting gave a pH value of 6.75, indicating a uniform hydrogen-ion concentration. Six composites of individual samples from three different spots in the first planting of the Austrian winter peas were more variable in reaction, the pH values ranging from 6.57 to 7.88. Obviously there was little need for a neutralizing agent in this soil.

## FIELD OBSERVATIONS

### GROWTH AND NODULATION

On January 31, 1931, plants grown from seed treated with cultures 510, 511, and 518 were observed to be greener than those in other plots of the first planting of Austrian winter peas, in some of which the plants were turning yellow and a few were dying. On the plots containing mostly poorly colored plants, an occasional rather vigorous plant was noticed. All plants, even those in the worst condition, had nodules on their roots. As in earlier studies (*9*) it was found that on the better plants the nodules tended to branch or become convolute, whereas on most of the poor plants the nodules were smaller and globular in shape, the former type being placed rather generally around the crown, and the latter scattered more or less evenly over the roots.

On February 17, 1931, a second examination of representative plants was made. This revealed much the same condition as the first. (Table 2.) At this time there was no evidence that either the basic slag or the lime had influenced the growth of the plants or the formation of nodules.

TABLE 2.—*Growth and nodulation of Austrian winter peas and other legumes at Jeanerette, La., February 17, 1931*

| Name of plant [a] | Inoculation treatment | Growth | Nodulation |
|---|---|---|---|
| Canada field pea | 510 | Excellent | Normal.[b] |
| Alaska garden pea | None | Fair | Scattered. |
| Hairy vetch | None | Poor | Plentiful. |
| Austrian winter pea (2) | 512 | Excellent | Normal. |
| Austrian winter pea | 484 | Poor | Scarce. |
| Do | 484 | Fair | Do. |
| Do | 518 | Poor | Do. |
| Austrian winter pea (2) | 518 | Excellent | Normal. |
| Austrian winter pea (3) | 511 | do | Do. |
| Austrian winter pea (6) | None | Poor | Scarce. |
| Austrian winter pea (2) | 510 | Excellent | Normal. |
| Austrian winter pea (3) | 513 | Poor | Scarce. |

[a] Numbers in parentheses indicate the number of samples examined.
[b] Normal means tendency to branch and locate near the crown, all other notations in this column refer to scattered globular nodules.

Plants of Austrian winter pea from seed inoculated with culture 510 accidentally mixed with the seed of hairy vetch were very good,

and plants from the latter were much better than other vetch plants from untreated seed, most of which did not exceed 4 inches in height. All vetch plants had nodules. Whether the beneficial effect of the association of vetch with Austrian winter pea was due to a transference of adapted organisms or to the utilization of by-products is not known, but it is an interesting lead to consider. The seed of Canada field peas and Alaska garden peas did not germinate satisfactorily, and the crop that was made was not satisfactory.

A better picture of the differences between excellent and poor growth and between the two types of nodulation is shown in Figure 2. Although some of the roots were broken off in removing the gummy soil and many nodules are believed to have been lost in the washing process, Figure 2 adequately illustrates the extreme variation in root system and type of nodulation resulting from treatments with beneficial and nonbeneficial cultures. No differences were observed between the untreated plants and roots and those treated with nonbeneficial cultures.

The Austrian-winter-pea plants from the ridged beds were better than those from the flat bed, but not nearly so good as plants from the areas inoculated with good cultures. Possibly the ridging limited the water supply and thereby interfered with the activities of the native nonbeneficial nodule bacteria.

During the course of the experiment, more especially in the later stages, some of the poor plants which survived the earlier effects of treatment took on renewed life. This is presumed to have been due to the spreading of good nodule bacteria by means of dust, implements, insects, man, or beast. It is reasonable to expect, and experience offers a slight basis for believing, that infections may occur with bacteria of good, bad, and intermediate efficiency, so it is quite possible that where there is an apparent turn for the better, the more efficient organisms may have obtained the upper hand or the progeny of the nonbeneficial organisms may have become better adapted to the plant while within the nodule.

## CROP YIELDS

By harvest time a great many of the vigorous growing Austrian-winter-pea plants had lost a rather large proportion of their lower leaves by shedding or decay. Plant lice may have contributed to the losses, since they were plentiful on the plants of the early sowing. The pea vines, which had grown over the dividing furrows, were thrown back on their respective plots before harvesting. The vines were cut with a scythe during rather dry weather and weighed promptly after cutting.

The larger weeds were separated from the cut material, but it was not practical to remove all the little weeds and grass that had grown up in the plots where pea plants had died. It is to be expected that the weight of the grass and weeds will partly compensate for the dead pea plants and may therefore give a false picture in the tabulation of yield data. Where the growth of the winter peas was good and the vines thoroughly covered the ground, weeds and grass were largely suppressed.

FIGURE 2.—Representative Austrian winter-pea plants from plots treated with a beneficial nodule culture (right), and with a nonbeneficial nodule culture (left)

TABLE 3.—*Yields of Austrian winter peas from plots inoculated with a beneficial culture and from untreated plots*

[Planted October 22, 1930, harvested April 24, 1931]

| Inoculation treatment | Yield of hay from 10 by 20 foot plot | | Moisture in green pea vines | Inoculation treatment | Yield of hay from 10 by 20 foot plot | | Moisture in green pea vines |
|---|---|---|---|---|---|---|---|
| | Green | Moisture free | | | Green | Moisture free | |
| | *Pounds* | *Pounds* | *Per cent* | | *Pounds* | *Pounds* | *Per cent* |
| 511 | 81 | 13.98 | 82.74 | None | 6 | 1.05 | 82.56 |
| None | 21 | 4.35 | 79.28 | 511 | 79 | 19.97 | 74.72 |
| 511 | 86 | 26.55 | 69.13 | None | 16 | 3.35 | 79.04 |

Table 3 shows the yields from uninoculated Austrian winter peas compared with those from peas inoculated with a beneficial culture. The data indicate an increase of 485 per cent in the yield from the inoculated plots as compared with that from the uninoculated. If such improvement can be made fairly consistently in these soils, it is apparent that the addition of the proper organisms will mean the difference between success and failure.

A comparison of the effects of basic slag and lime on Austrian winter peas receiving different cultures is given in Table 4. The data shown in Table 4 indicate no decided superiority in crop yield as a result of the application of basic slag or lime.

TABLE 4.—*Plot yields in pounds (green weight) of Austrian winter-pea hay per 10 by 20 foot plots, as affected by inoculation, basic slag, or lime*

[Planted October 22, 1930; harvested, except as otherwise noted, May 11, 1931]

| Inoculation treatment | Yield with— | | | Total yield |
|---|---|---|---|---|
| | Lime | Basic slag | No addition | |
| 512 | 80 | 92 | 75 | 247 |
| 510 | 73 | 86 | 71 | 230 |
| None | 61 | 61 | 50 | 172 |
| 513 | 44 | 55 | 66 | 165 |
| 473 | 43 | 54 | 45 | 142 |
| None | 29 | 33 | 53 | 115 |
| Total | 330 | 381 | 360 | |
| 484 | 31 | 45 | 60 | 136 |
| None | 35 | 38 | 44 | 117 |
| 511 | 62 | 72 | 57 | 191 |
| 518 | 55 | 76 | 63 | 194 |
| None | 42 | 45 | 47 | 134 |
| 515 | 46 | 59 | 55 | 160 |
| Total | 271 | 335 | 326 | |
| None | 62 | 59 | 51 | 172 |
| 513 | 47 | 52 | 41 | 140 |
| 473 | 50 | 45 | 36 | 131 |
| None | 56 | 52 | 44 | 152 |
| 510 | 47 | 59 | 48 | 154 |
| 512 | 53 | 62 | 58 | 173 |
| Total | 315 | 329 | 278 | |

TABLE 4.—*Plot yields in pounds (green weight) of Austrian winter-pea hay per 10 by 20 foot plots, as affected by inoculation, basic slag, or lime*—Continued

| Inoculation treatment | Yield with— | | | Total yield |
|---|---|---|---|---|
| | Lime | Basic Slag | No addition | |
| 511 | 56 | 59 | 63 | 178 |
| 518 | 64 | 51 | 51 | 166 |
| None | 50 | 50 | 36 | 136 |
| 515 | 47 | 43 | 30 | 120 |
| 484 | 28 | 29 | 16 | 73 |
| None | 43 | 45 | 27 | 115 |
| Total | 288 | 277 | 223 | |
| 473 | 47 | 48 | 44 | 139 |
| None | 44 | 45 | 43 | 132 |
| 510 | 50 | 55 | 43 | 148 |
| 512 | 51 | 55 | 49 | 155 |
| None | 19 | 18 | 20 | 57 |
| 513 | 31 | 31 | 30 | 92 |
| Total | 242 | 252 | 229 | |
| None | 62 | 59 | 63 | 184 |
| 515 | 42 | 44 | 46 | 132 |
| 484 | 37 | 45 | 42 | 124 |
| None | 16 | 6 | 21 | 43 |
| 511 [a] | 79 | 86 | 81 | 246 |
| 518 [a] | 42 | 47 | 37 | 126 |
| Total | 278 | 287 | 290 | |
| Grand total | 1,724 | 1,861 | 1,706 | |

[a] Harvested Apr. 24, 1931.

Table 5 shows the relative value of the different inoculation cultures.

TABLE 5.—*Summary of the hay-crop yields of Austrian winter peas from planting made October 22, 1930, and harvested May 11, 1931*

| Inoculation treatment | Plots | Average yield of dry hay | Inoculation treatment | Plots | Average yield of dry hay |
|---|---|---|---|---|---|
| | *Number* | *Pounds* | | *Number* | *Pounds* |
| None | 36 | 42.4 | 518 | 9 | 54.0 |
| 473 | 9 | 45.8 | 511 | 6 | 61.5 |
| 513 | 9 | 44.1 | 484 | 9 | 37.0 |
| 510 | 9 | 59.1 | 515 | 9 | 47.3 |
| 512 | 9 | 63.9 | | | |

Even with the larger proportion of weeds and grass in the areas inoculated with nonbeneficial bacteria, there is apparently an appreciable increment in crop yield due to cultures 510, 512, 518, and 511, whereas cultures 473, 513, 484, and 515 are not greatly superior to no treatment.

The second planting of Austrian winter peas was substantially the same in layout as the first, yet the results were not the same. The whole crop, regardless of treatment, gave no significant differences attributable to basic slag, lime, or inoculation. (Table 6.) The total yield of dry hay from the 36 plots was as follows: 1,624 pounds from lime, 1,680 pounds from basic slag, and 1,616 pounds from no treat-

ment. The lack of variation in yields from the second planting may have been due to a scattering of good types of nodule organisms by an inundation.

TABLE 6.—*Average yields of dry hay from plots of the second planting of Austrian winter peas, sown November 3, 1930, and harvested May 22, 1931*

| Inoculation treatment | Plots | Yield of dry hay | | Inoculation treatment | Plots | Yield of dry hay | |
|---|---|---|---|---|---|---|---|
| | | Total | Average per plot | | | Total | Average per plot |
| | *Number* | *Pounds* | *Pounds* | | *Number* | *Pounds* | *Pounds* |
| None | 36 | 1,680 | 46.7 | 511 | 9 | 444 | 49.3 |
| 473 | 9 | 367 | 40.8 | 484 | 9 | 400 | 44.4 |
| 513 | 9 | 387 | 43.0 | 515 | 9 | 411 | 45.7 |
| 510 | 9 | 396 | 44.0 | | | | |
| 512 | 9 | 382 | 42.4 | Total or mean | 108 | 4,920 | 45.5 |
| 518 | 9 | 453 | 50.3 | | | | |

## NITROGEN IN AUSTRIAN WINTER PEA AS AFFECTED BY INOCULATION

Samples of pure winter-pea hay from a few plots were harvested February 16, 1931, dried thoroughly, and ground for analysis. The results are given in Table 7.

Comment on these results is almost unnecessary since they are of the same general nature as the other evidence given of the effect of nodule organisms on Austrian winter peas.

TABLE 7.—*Nitrogen analyses of field-grown Austrian winter-pea hay harvested at Jeanerette, La., February 16, 1931*

[Planted October 22, 1930]

| Inoculation treatment | 10 plants (dry weight) | Nitrogen | Total nitrogen |
|---|---|---|---|
| | *Grams* | *Per cent* | *Grams* |
| None | 5.3 | 0.94 | 0.05 |
| 511 | 37.25 | 3.40 | 1.27 |
| 513 | 13.46 | .86 | .12 |
| 518 | 40.77 | 3.08 | 1.25 |

## GREENHOUSE EXPERIMENTS

In a series of greenhouse experiments at Rosslyn, Va., Austrian winter peas were grown in sterilized sand moistened with Bryan-Crone's nutrient solution (*2*). Before the seed was sown it was sterilized with 1:500 mercuric chloride, washed thoroughly with sterile tap water, and inoculated with bacteria from test-tube cultures, the usual precautions being taken to prevent the transfer of nodule bacteria from one series to another. Each treatment was replicated 10 times and each replication usually consisted of about five plants. The data are shown in Table 8.

TABLE 8.—*Nitrogen fixation in greenhouse plantings of Austrian winter peas*

| Inoculation treatment | Duration of experiment | Nodules per plant | Dry weight (50 plants) | Nitrogen | Total nitrogen |
|---|---|---|---|---|---|
| | | *Average number* | *Grams* | *Per cent* | *Gram* |
| None | Oct. 28, 1929 to Dec. 12, 1929 (45 days) | | 13.00 | 2.60 | 0.339 |
| 473 | | | 12.50 | 3.33 | .416 |
| 484 | | | 12.50 | 2.38 | .300 |
| 510 | | | 15.50 | 3.04 | .471 |
| 511 | | | 23.00 | 2.94 | .675 |
| 512 | | | 21.50 | 3.08 | .664 |
| 513 | | | 21.50 | 2.15 | .461 |
| 515 | | | 23.00 | 2.40 | .550 |
| 518 | | | 20.50 | 3.09 | .632 |
| None | Feb. 14, 1931 to Apr. 8, 1931 (53 days) | 3 | 15.60 | 1.30 | .203 |
| 510 | | 15 | 18.50 | 2.86 | .532 |
| 511 | | 16 | 20.75 | 2.74 | .568 |
| 513 | | 96 | 15.90 | 1.22 | .194 |
| 518 | | 13 | 26.85 | 2.36 | .635 |
| None | Apr. 4, 1931 to May 25, 1931 (51 days) | 1 | 12.80 | 1.72 | .220 |
| 512 | | 15 | 13.80 | 2.70 | .372 |
| 513 | | 135 | 13.20 | 2.04 | .269 |
| 548 | | 90 | 11.40 | 1.66 | .189 |
| 549 | | 99 | 12.20 | 1.68 | .205 |
| 550 | | 122 | 11.60 | 1.66 | .192 |
| 512+513 | | 29 | 11.70 | 1.94 | .227 |
| 513+$NaNO_3$ | | 44 | 14.90 | 2.12 | .316 |

In the first experiment, culture 510 apparently gave poor results, so far as dry weight and total nitrogen are concerned, yet the percentage of nitrogen rather closely parallels that of the other beneficial cultures. Considering matters on the basis of total nitrogen apparently fixed by nodule organisms, it will be noted that the plants from only one culture (484) contained less nitrogen than the control. Apparently the so-called nonbeneficial cultures were slightly beneficial when the quantity of nitrogen fixed is considered. However, in the second experiment the nitrogen fixed by the nonbeneficial culture (513) was actually less than that of the control and much less than that of any of the three beneficial cultures employed, and in the last experiment this nonbeneficial culture was slightly better than the control. When the two types of organisms (512 and 513) were used together the total nitrogen fixed was not greater than that fixed by the nonbeneficial organism alone. However, the addition of 0.1 per cent sodium nitrate to the solution originally used to moisten the sand (subsequent waterings being made without nitrogen additions) helped the plants inoculated with a nonbeneficial organism considerably.

## SOURCE OF THE NONBENEFICIAL ORGANISMS

The fact is evident that nodule bacteria beneficial and detrimental to Austrian winter peas exist in some of the soils around Jeanerette, La., the nonbeneficial ones evidently predominating. In seeking an explanation for the presence of these organisms it was found that plants of Louisiana vetch (*Vicia ludoviciana* Nutt.) grew plentifully and luxuriantly around the fields and in waste places. The roots of these wild plants are well populated with nodules. It is therefore reasonable to believe that the organisms causing these nodules are abundantly present in the soil. It is customary to consider that the organisms of the common vetch will cross-inoculate with garden

peas, field peas, Austrian winter peas, and other legumes, although data concerning the group relations of Louisiana vetch have not been worked out. However, acting on the supposition that the bacteria of this legume are related to those of the Austrian winter pea but not satisfactorily adapted to the latter, the writers made isolations from nodules for comparison with the nonbeneficial bacteria obtained from Austrian winter peas.

After the cultures from Louisiana vetch were purified they were applied to Austrian winter pea and Louisiana vetch seed in the greenhouse under controlled conditions, as indicated in Table 8. These cultures produced nodules on their specific hosts. The results with Austrian winter peas indicate that these Louisiana vetch nodule organisms produce the same effect as those of the nonbeneficial type whose effects have been previously noted in this paper. From April 4 to May 25, 1931, the Austrian winter-pea plants grown from seed treated with three cultures of nodule organisms of Louisiana vetch had much the same appearance as those from seed treated with a nonbeneficial culture isolated from Austrian winter pea nodules. (Fig. 3.) As compared with the control, Louisiana vetch organisms (548, 549, and 550) contributed no nitrogen to the Austrian winter peas; in fact they caused a slight loss. (Table 8.)

Nodules produced by Louisiana vetch cultures were plentiful, scattered, and globular. Nodules of this type are shown in Figures 4 and 5 in comparison with nodules produced by beneficial and nonbeneficial cultures from Austrian winter peas. Characteristic nodule assemblies are more distinct in greenhouse samples than in field samples. (Fig. 2.) Table 8 shows that the average number of nodules per plant is considerably higher when poor cultures of Austrian winter peas and Louisiana vetch were used than when beneficial organisms were used. The close similarity of response of Austrian winter peas to nodule organisms from Louisiana vetch and from apparently parasitized Austrian winter peas is indicative of the origin of the unadapted strains present in the soils studied.

## SUMMARY

Cultures of nodule bacteria from poor and vigorous Austrian winter-pea (*Pisum arvense*) plants from Jeanerette, La., have been compared with nodule bacteria from closely related legumes in field and greenhouse tests.

Drainage obtained by ridging the soil had but little effect, and lime and basic slag had practically no effect on the crop produced.

A favorable well-defined effect in quantity and quality of crop was evident in Austrian winter peas treated with efficient nodule bacteria in soil known to contain inefficient nodule-producing organisms.

Evidence has been presented which shows that the nodule organisms of the Louisiana vetch (*Vicia ludoviciana*) reduce the yield of Austrian winter pea on account of their unadaptability to this host.

172087—33——6

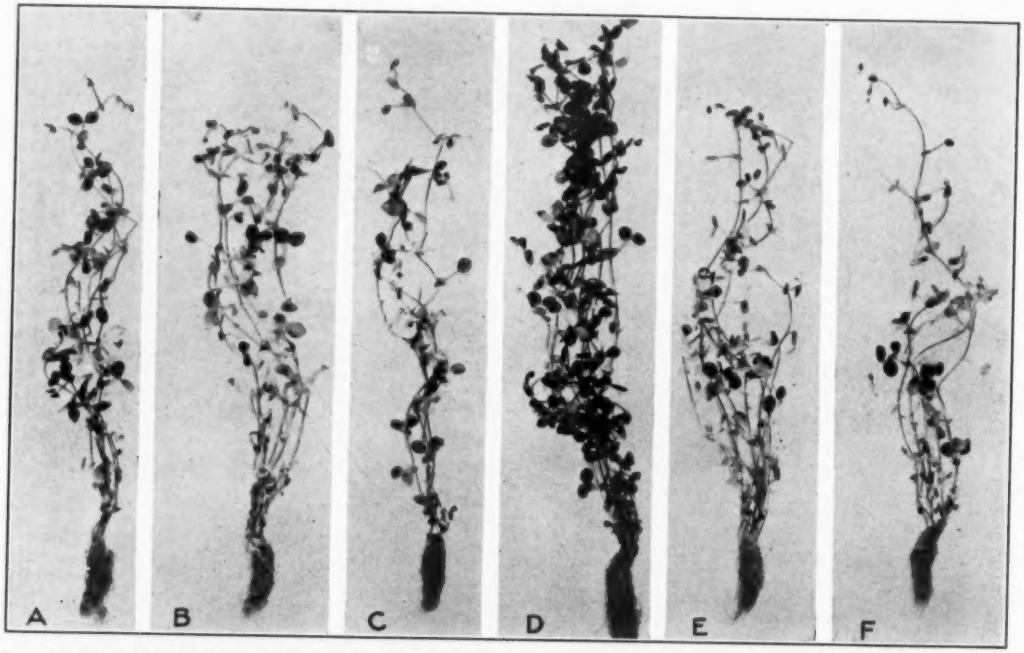


FIGURE 3.—Typical single-pot clumps of Austrian winter-pea plants treated with nodule organisms as follows: A, None; B, nonbeneficial culture 513; C, Louisiana vetch culture 548; D, beneficial culture 512; E and F, Louisiana vetch cultures 549 and 550

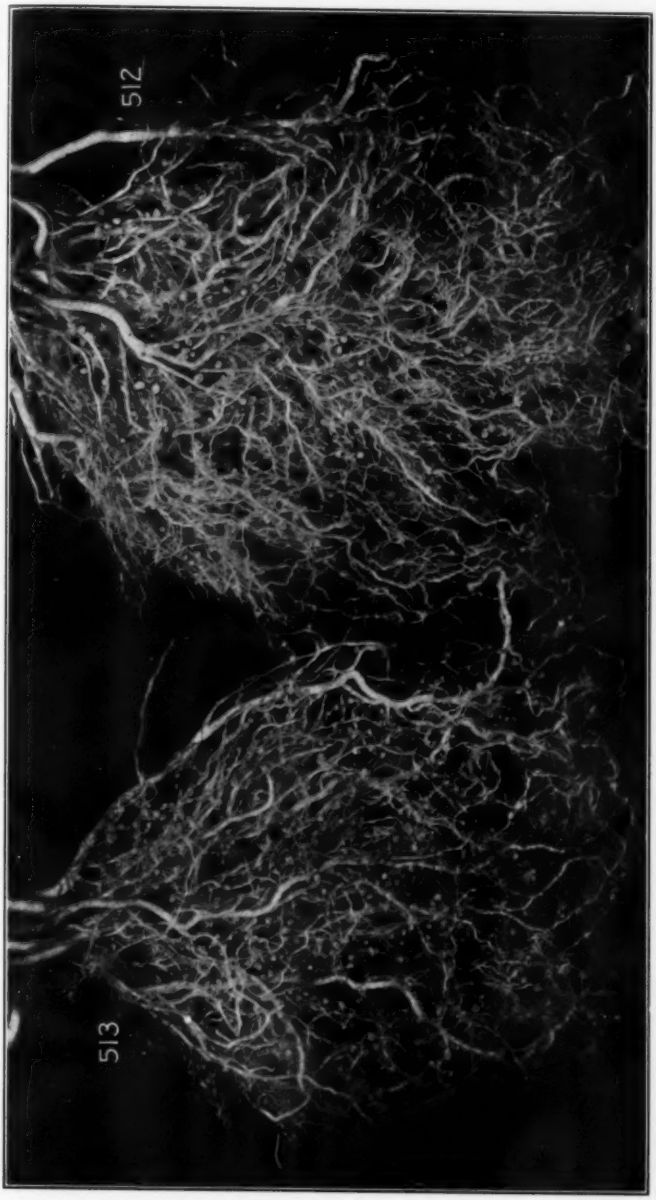


FIGURE 4.—Beneficial culture 512 and nonbeneficial culture 513 on Austrian winter peas grown in greenhouse

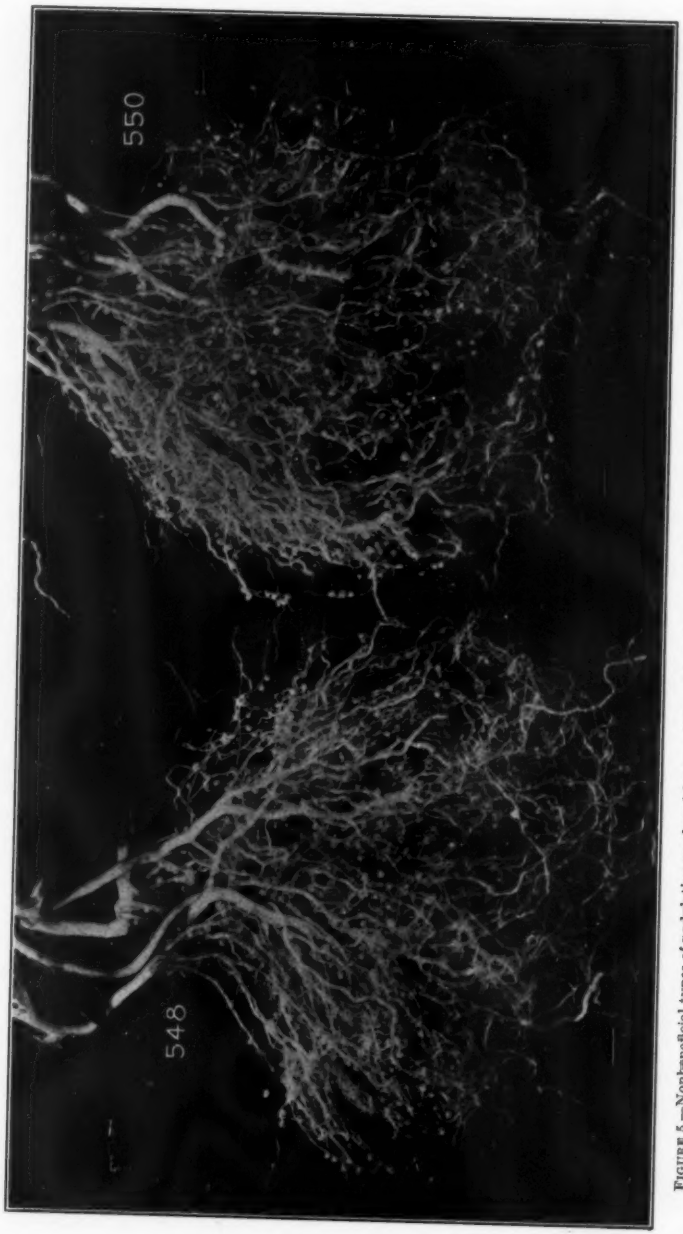


FIGURE 5.—Nonbeneficial types of nodulation produced by two different isolations from nodules of *Vicia ludoviciana* on Austrian winter pea in the greenhouse

## LITERATURE CITED


(1) ALLEN, O. N., and BALDWIN, I. L.
1931. THE EFFECTIVENESS OF RHIZOBIA AS INFLUENCED BY PASSAGE THROUGH THE HOST PLANT. Wis. Agr. Expt. Sta. Research Bul. 106, 56 p., illus.

(2) BRYAN, O. C.
1922. EFFECT OF DIFFERENT REACTIONS ON THE GROWTH AND NODULE FORMATION OF SOYBEANS. Soil Sci. 13: 271–302, illus.

(3) DUNHAM, D. H., and BALDWIN, I. L.
1931. DOUBLE INFECTION OF LEGUMINOUS PLANTS WITH GOOD AND POOR STRAINS OF RHIZOBIA. Soil Sci. 32: 235–249, illus.

(4) EATON, S. V.
1931. EFFECTS OF VARIATION IN DAY-LENGTH AND CLIPPING OF PLANTS ON NODULE DEVELOPMENT AND GROWTH OF SOYBEAN. Bot. Gaz. 91: 113–143, illus.

(5) HELZ, G. E., BALDWIN, I. L., and FRED, E. B.
1927. STRAIN VARIATIONS AND HOST SPECIFICITY OF THE ROOT-NODULE BACTERIA OF THE PEA GROUP. Jour. Agr. Research 35: 1039–1055, illus.

(6) HILTNER, L.
1900. UEBER DIE URSACHEN, WELCHE DIE GRÖSSE, ZAHL, STELLUNG UND WIRKUNG DER WURZELKNÖLLCHEN DER LEGUMINOSEN BEDINGEN. Arb.–Biol. Anst. Land u. Forstw. 1: 177–222, illus.

(7) JONES, J. W.
1926. CONCERNING THE GROWTH OF MUNG BEANS ON SUBMERGED LAND. Jour. Amer. Soc. Agron. 18: 366–367.

(8) LEONARD, L. T.
1926. A PRELIMINARY NOTE ON THE RELATION OF PHOTOSYNTHETIC CARBOHYDRATE TO NODULE FORMATION ON SOYBEANS. Jour. Amer. Soc. Agron. 18: 1012–1016.

(9) ———
1930. A FAILURE OF AUSTRIAN WINTER PEAS APPARENTLY DUE TO NODULE BACTERIA. Jour. Amer. Soc. Agron. 22: 277–279.

(10) SMITH, E. F.
1905–1914. BACTERIA IN RELATION TO PLANT DISEASES. 3 v., illus. Washington, D. C. (Carnegie Inst. Wash. Pub. 27.)

(11) THORNTON, H. G.
1930. THE INFLUENCE OF THE HOST PLANT IN INDUCING PARASITISM IN LUCERNE AND CLOVER NODULES. Roy. Soc. [London], Proc., Ser. B 106: 110-122, illus.

(12) VOORHEES, E. B., and LIPMAN, J. G.
1907. A REVIEW OF INVESTIGATIONS IN SOIL BACTERIOLOGY. U. S. Dept. Agr., Off. Expt. Stas. Bul. 194, 108 p.

(13) WORONINE, M.
1867. OBSERVATIONS SUR CERTAINES EXCROISSANCES QUE PRÉSENTENT LES RACINES DE L'AUNE ET DU LUPIN DES JARDINS. Ann. Sci. Nat., Bot. (5) 7: [73]–86, illus.

(14) WUNCHIK, H.
1925. ERHÖHUNG DER WIRKSAMKEIT DER KNÖLLCHENERREGER UNSERER SCHMETTERLINGSBLÜTLER DURCH PASSIERIEN DER WIRTSPFLANZE. Centbl. Bakt. [etc.] (II) 64: 395–445, illus.

# CHEMICAL COMPOSITION OF NATIVE ALASKAN HAYS HARVESTED AT DIFFERENT PERIODS OF GROWTH[1]

By RUTH G. CAPEN, *Junior Chemist*, and J. A. LECLERC, *Senior Chemist, Food Research Division, Chemical and Technological Research, Bureau of Chemistry and Soils, United States Department of Agriculture*

## INTRODUCTION

Alaska is handicapped agriculturally chiefly because of its very short growing season. However, some common vegetables, small fruits, cereals, and root crops are now being grown in many parts of Alaska, and certain native forage plants seem to thrive there. Most of these forage plants have a potential economic value. It was therefore deemed desirable to study the chemical composition of some of them cut at different stages of growth and to compare them with other forage plants cultivated in Alaska.

## PREVIOUS INVESTIGATIONS

In 1904 the Bureau of Chemistry (*4, p. 276*)[2] made analyses for the Alaska station of three native grasses which were cut when in flower. The results are given in Table 1. Extensive investigations of this general nature have been conducted at certain State experiment stations. Knight, Hepner, and Nelson (*7*), of the Wyoming Agricultural Experiment Station, studied the chemical composition of forage plants grown at different altitudes (where the growing season is short) and harvested at the same stage of growth. Hepner (*5*), also of the Wyoming station, investigated the relation of the nitrogen content of soils to the nitrogen content of grasses grown at high altitudes. Cundy (*3*) and Robert (*8*) continued these investigations on forage plants. The former studied the variation in the chemical composition of samples collected at different periods during the haying season, and the latter studied the effect of altitude, seasonal variation, and shade on their chemical composition. Hopper and Nesbitt (*6*), of the North Dakota Agricultural Experiment Station, likewise analyzed native prairie grasses at several stages of growth.

TABLE 1.—*Composition (percent) of Alaskan forage plants*[a]

| Plant | Moisture | Ash | Ether extract | Crude fiber | Proteids | Nitrogen-free extract |
|---|---|---|---|---|---|---|
| Bluetop (*Calamagrostis langsdorfii*) | 7.18 | 3.90 | 1.03 | 42.94 | 4.58 | 40.37 |
| Sedge (*Carex cryptocarpa*) | 5.85 | 10.65 | 2.12 | 25.72 | 10.32 | 45.34 |
| Beach rye (*Elymus mollis*) | 11.92 | 7.51 | 2.26 | 30.31 | 12.71 | 35.29 |

[a] Georgeson, C. C. (*4, p. 276*).

[1] Received for publication Aug. 31, 1932; issued May 1933.

[2] Reference is made by number (italic) to Literature Cited, p. 668.

## PRESENT INVESTIGATION

Sixteen samples each of native sedge (*Carex* sp.), redtop (*Calamagrostis* sp.), and cotton grass (*Eriophorum* sp.) were recently submitted to this bureau by the Office of Experiment Stations for analysis, together with one sample each of native vetch (*Vicia cracca*), yellow-flowered alfalfa (*Medicago falcata*), and bromegrass (*Bromus inermis*) to be used for comparison. These were all forage plants grown at the Alaska Agricultural Experiment Station at Fairbanks. The nomenclature has not been checked. The sedge, redtop, and cotton grass were sampled or harvested at intervals of one week from July 22 to November 4, 1929, and the vetch, alfalfa, and bromegrass were harvested on July 19, 1929.

The report of the Alaska Agricultural Experiment Stations (*1*) shows that during April, May, and June the weather was comparatively dry and cool, and plants grew slowly. During the second week of July there was a heavy rainfall, and growth was vigorous during the remainder of the month. August and the first part of September were cool, cloudy, and wet, causing crops to mature slowly, and harvesting in general was delayed until the last week in September.

The plants were air-dried when received. They were ground and analyzed for moisture, ash, nitrogen, fat, fiber, and sugars. The analytical methods used were those outlined by the Association of Official Agricultural Chemists (*2, p. 277*). The time of harvesting and the results of the analyses are shown in Table 2.

The protein content of the native forage plants was highest in the third cutting, that of August 5, and in the main decreased as the season advanced.

The fiber content gradually increased in all cases as the stage of growth advanced. This is especially true of redtop.

The percentage of ash in sedge was fairly constant, increasing slightly until the last three cuttings, or the middle of October, after which it dropped appreciably. The ash content of redtop remained fairly high throughout the entire period of growth; in fact, it was greater in the last cuttings than in the early ones. The ash of cotton grass was low and decreased as the season advanced, being similar in this respect to the ash of sedge.

The percentage of fat (ether extract) remained fairly constant in all samples harvested at different periods. In the sedge, the three latest cuttings had a slightly higher fat content than the early cuttings, whereas in redtop the four earliest were higher in fat than the subsequent cuttings; cotton grass was more or less variable throughout.

The percentage of sugars was uniformly higher in the early cuttings. The late cuttings contained from 50 to 20 per cent of the amount of sugar found in the early cuttings.

The results show that at the same period of harvesting, the native sedge, redtop, and cotton grass compare favorably with vetch and bromegrass in protein content, but are considerably lower in this constituent than is the yellow-flowered alfalfa.

The fiber content of cotton grass and redtop agrees fairly well with that of alfalfa and bromegrass. The fiber of sedge is the lowest of all, and that of vetch is the highest.

TABLE 2.—*Composition (per cent) of samples of Alaskan forage plants cut at various stages of growth*

NATIVE SEDGE (CAREX SP.)

| Date cut (1929) | Moisture | Ash | Nitrogen | Protein | Fat | Crude fiber | Total sugar |
|---|---|---|---|---|---|---|---|
| July 22 | 5.26 | 4.36 | 1.76 | 11.00 | 1.79 | 28.95 | 3.82 |
| July 29 | 5.41 | 4.72 | 1.31 | 8.19 | 1.74 | 32.71 | 5.42 |
| Aug. 5 | 5.38 | 4.66 | 1.96 | 12.25 | 1.51 | 30.70 | 2.17 |
| Aug. 12 | 4.98 | 4.34 | 1.74 | 10.88 | 2.17 | 30.00 | 3.40 |
| Aug. 19 | 4.98 | 4.38 | 1.57 | 9.81 | 1.88 | 29.32 | 5.25 |
| Aug. 26 | 4.85 | 4.78 | 1.32 | 8.25 | 2.02 | 25.55 | 5.70 |
| Sept. 2 | 5.08 | 4.49 | 1.25 | 7.81 | 2.56 | 34.59 | 2.20 |
| Sept. 9 | 4.96 | 5.32 | 1.39 | 8.69 | 1.45 | 34.30 | 1.90 |
| Sept. 16 | 4.85 | 4.75 | 1.28 | 8.00 | 1.48 | 32.86 | 3.21 |
| Sept. 23 | 5.62 | 4.58 | .94 | 5.87 | 1.53 | 32.66 | ---------- |
| Sept. 30 | 5.18 | 4.66 | .91 | 5.69 | 1.34 | 34.12 | .59 |
| Oct. 7 | 5.54 | 5.58 | 1.01 | 5.31 | 1.56 | 35.87 | ---------- |
| Oct. 14 | 5.38 | 5.20 | .93 | 5.78 | 1.80 | 34.19 | .86 |
| Oct. 21 | 5.24 | 3.36 | .59 | 3.68 | 2.18 | 35.87 | .85 |
| Oct. 28 | 4.78 | 3.22 | .62 | 3.89 | 2.13 | 36.89 | .57 |
| Nov. 4 | 4.84 | 2.91 | .59 | 3.69 | 2.00 | 35.66 | .52 |

REDTOP (CALAMAGROSTIS SP.)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| July 22 | 5.33 | 4.47 | 1.42 | 8.88 | 1.78 | 32.85 | 3.17 |
| July 29 | 5.13 | 4.41 | 1.71 | 10.69 | 1.74 | 31.51 | 4.87 |
| Aug. 5 | 4.94 | 6.01 | 1.84 | 11.50 | 1.74 | 32.37 | 3.26 |
| Aug. 12 | 4.86 | 7.24 | .78 | 4.88 | 1.85 | 33.13 | 3.60 |
| Aug. 19 | 5.04 | 5.09 | 1.00 | 6.25 | 1.33 | 36.51 | 4.80 |
| Aug. 26 | 4.95 | 6.98 | .74 | 4.63 | 1.67 | 34.73 | 3.29 |
| Sept. 2 | 4.77 | 6.60 | .56 | 3.50 | 1.69 | 35.51 | 4.04 |
| Sept. 9 | 4.81 | 6.53 | .81 | 5.06 | 1.72 | 36.98 | 3.50 |
| Sept. 16 | 5.05 | 5.40 | .56 | 3.50 | 1.66 | 39.22 | 2.74 |
| Sept. 23 | 5.37 | 7.35 | .81 | 5.06 | 1.59 | 34.85 | 2.36 |
| Sept. 30 | 5.07 | 4.20 | .74 | 4.63 | 1.42 | 38.21 | .25 |
| Oct. 7 | 4.58 | 4.41 | .75 | 4.69 | 1.52 | 40.90 | .45 |
| Oct. 14 | 4.46 | 6.51 | .43 | 2.63 | 1.56 | 40.99 | .70 |
| Oct. 21 | 4.70 | 6.64 | .34 | 2.13 | 1.60 | 38.02 | .26 |
| Oct. 28 | 4.80 | 6.34 | .36 | 2.25 | 1.49 | 40.61 | .82 |
| Nov. 4 | 4.81 | 6.77 | .36 | 2.25 | 1.69 | 41.57 | .06 |

COTTON GRASS (ERIOPHORUM SP.)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| July 22 | 5.94 | 2.93 | 1.54 | 9.63 | 1.90 | 31.96 | 2.45 |
| July 29 | 5.52 | 2.17 | 1.47 | 9.19 | 1.59 | 31.45 | 5.88 |
| Aug. 5 | 5.90 | 2.57 | 1.64 | 10.19 | 1.44 | 33.28 | 2.40 |
| Aug. 12 | 5.62 | 2.48 | 1.57 | 9.81 | 1.38 | 32.30 | 3.54 |
| Aug. 19 | 5.77 | 2.51 | 1.42 | 8.88 | 1.50 | 29.89 | 4.01 |
| Aug. 26 | 5.98 | 2.50 | 1.40 | 8.75 | 1.86 | 27.77 | 5.73 |
| Sept. 2 | 6.00 | 2.11 | 1.06 | 6.62 | 2.01 | 32.85 | 3.95 |
| Sept. 9 | 5.44 | 1.80 | .90 | 5.63 | 2.01 | 34.85 | 3.01 |
| Sept. 16 | 5.55 | 2.03 | .87 | 5.44 | 1.89 | 32.24 | 3.42 |
| Sept. 23 | 5.48 | 1.72 | .74 | 4.63 | 1.92 | 33.38 | 1.71 |
| Sept. 30 | 5.64 | 1.63 | .78 | 4.88 | 1.91 | 35.30 | 2.28 |
| Oct. 7 | 5.55 | 1.90 | .92 | 5.75 | 1.92 | 33.45 | 2.72 |
| Oct. 14 | 5.80 | 1.68 | .52 | 3.25 | 1.68 | 33.07 | 1.78 |
| Oct. 21 | 5.85 | 1.97 | .64 | 4.00 | 1.77 | 33.44 | 1.47 |
| Oct. 28 | 5.86 | 1.91 | .67 | 4.19 | 1.76 | 33.88 | 1.75 |
| Nov. 4 | 5.69 | 1.79 | .64 | 4.00 | 1.54 | 34.63 | 1.20 |

VETCH (VICIA CRACCA)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| July 19 | 6.60 | 6.65 | 1.71 | 10.69 | 1.40 | 35.54 | 1.32 |

YELLOW-FLOWERED ALFALFA (MEDICAGO FALCATA)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| July 19 | 6.72 | 7.22 | 2.48 | 16.50 | 1.34 | 32.96 | 2.26 |

BROMEGRASS (BROMUS INERMIS)

| | | | | | | | |
|---|---|---|---|---|---|---|---|
| July 22 | 5.51 | 7.29 | 1.22 | 7.63 | 1.38 | 32.71 | 5.76 |

Vetch, bromegrass, and alfalfa are all considerably higher in ash and appreciably lower in fat than are cotton grass, sedge, and redtop. Sugar content is highest in bromegrass and lowest in vetch; the sugar content of alfalfa is about the same as that of cotton grass, but considerably lower than that of sedge and redtop.

## SUMMARY AND CONCLUSIONS

Fifty-one samples of forage plants grown in Alaska in 1929 were analyzed for moisture, ash, crude protein, fat, fiber, and sugar.

The percentage of protein decreased as the season advanced. Redtop especially showed a markedly low protein content after the early stages of growth.

The percentage of fiber increased in all cases as the season advanced.

The ash content of sedge and redtop was higher than that of cotton grass at every stage of growth. The ash content of redtop was higher in the late cuttings than in the earliest ones. The reverse was true in the case of cotton grass and sedge.

The fat content of sedge and cotton grass was slightly higher than that of redtop.

The native plants examined were richer in sugar (at the same stage of growth) than the introduced plants, with the exception of bromegrass.

The native forage plants of Alaska on the whole compare favorably with vetch and bromegrass but not with yellow-flowered alfalfa.

According to these analyses the feeding values of the native hays decrease rapidly after August, as is shown by the decrease in protein and the increase in fiber. Therefore it would seem advisable to harvest these varieties late in August or early in September.

## LITERATURE CITED

(1) Alberts, H. W.
1930. Reports of the Alaska Agricultural Experiment Stations, 1929. Alaska Agr. Expt. Sta. Rpt. 1929, 58 p., illus.

(2) Association of Official Agricultural Chemists.
1930. Official and tentative methods of analysis. ... Ed. 3, 593 p., illus. Washington, D. C.

(3) Cundy, A. T.
1924. Wyoming forage plants and their chemical composition—studies no. 6. Wyo. Agr. Expt. Sta. Bul. 137, 16 p., illus.

(4) Georgeson, C. C.
1905. Report of C. V. Piper on the grazing lands of the south Alaska coast and their possible utilization. Alaska Agr. Expt. Sta. Ann. Rpt. 1904 : 269–285.

(5) Hepner, F. E.
1918. Wyoming forage plants and their chemical composition—studies no. 5. Wyo. Agr. Expt. Sta. Ann. Rpt. 28 : [116]–[128].

(6) Hopper, T. H., and Nesbitt, L. L.
1930. Chemical composition of some North Dakota pasture and hay grasses. N.Dak. Agr. Expt. Sta. Bul. 236, 38 p., illus.

(7) Knight, H. G., Hepner, F. E., and Nelson, A.
1905–11. Wyoming forage plants and their chemical composition—studies 1 to 4. Wyo. Agr. Expt. Sta. Buls. 65, 70, 76, and 87, illus.

(8) Roberts, E. N.
1926. Wyoming forage plants and their chemical composition—studies no. 7. effect of altitude, seasonal variation and shading experiments. Wyo. Agr. Expt. Sta. Bul. 146, p. [35]–89, illus.

# A CHEMICAL STUDY OF ENSILING SOYBEANS[1]

By W. M. NEAL, *Associate in Animal Nutrition*, and R. B. BECKER, *Specialist in Dairy Husbandry, Florida Agricultural Experiment Station*

## INTRODUCTION

In the course of an investigation of the feeding value of soybean silage, observations were made upon the normal changes that occur in soybeans during the ensiling process. These observations were made in an effort to determine the efficiency of the silo in preserving the feed nutrients of a legume roughage. Such information is of particular importance in regions where seasonal rainfall ordinarily prevents the satisfactory curing of hay.

## WORK OF OTHER INVESTIGATORS

Soybeans (*Soja max*) have been investigated extensively as a combination silage crop planted in conjunction with corn, cane, and other erect-growing forage crops, but only rarely have they been ensiled alone. Hills (*4*)[2] ensiled 564 pounds of green soybeans between layers of corn, and found that they produced a pleasant aromatic silage palatable to cattle. Eckles (*3*) mentioned losses as arising from surface exposure, mold, and the normal fermentation processes in the silo. These investigators, as well as Woll and Humphrey (*9*), reported comparative analyses of fresh soybeans and the silage made from them.

## PLAN OF THE INVESTIGATION

In each of three consecutive years (1929, 1930, and 1931) a silo 10 feet in diameter by 20 feet 10 inches in height was filled with soybeans cut in the early bloom stage, giving about 15 feet of settled silage. As the filling progressed large samples were taken, and 1 kg of each was oven dried for chemical analysis. Ten kilograms were placed in a muslin bag and returned to the silo. Five such bags were deposited at various depths in the center of the silo each year. Each bag was covered with a layer of silage over which a dampened piece of burlap was placed to aid in locating it when the silage was being removed.

When removed, the contents of each bag were weighed, and 1 kg was oven dried for chemical analysis. By a computation of the quantity of each constituent determined, both in fresh soybeans and in the corresponding sample bag, changes in composition incident to the ensiling process were determined. In making the determinations surface spoilage was not considered, its importance being related to the height of the silo.

Proximate analyses were made by the methods of the Association of Official Agricultural Chemists (*1*); and calcium, magnesium, and phosphorus by the method of Morris, Nelson, and Palmer (*5*).

[1] Received for publication Oct. 18, 1932; issued May, 1933.
[2] Reference is made by number (italic) to Literature Cited, p. 673.

## PRESENTATION AND DISCUSSION OF RESULTS

Fifteen parallel samples of fresh soybeans and of soybean silage were analyzed. The averages of these analyses are presented in Table 1. The analyses of other workers, computed to the dry-matter basis, are given for comparison. In general, these latter indicate a decrease in the nitrogen-free extract, slight change in the protein content, a relative increase in crude fiber and ash, and apparently an increase in ether-soluble compounds arising from the end products of the fermentation processes in the silo.

TABLE 1.—*Composition (percentage) of green soybeans and of soybean silage, as reported by the Florida, Missouri, Vermont, and Wisconsin Experiment Stations*

| Source and class of feed | Total moisture | Composition on moisture-free basis | | | | |
|---|---|---|---|---|---|---|
| | | Crude protein | N-free extract | Crude fiber | Ether extract | Ash |
| Florida: [a] | | | | | | |
| Fresh soybeans | 74.61 | 12.12 | 40.00 | 36.38 | 2.41 | 9.07 |
| Soybean silage | 76.94 | 8.77 | 36.35 | 41.10 | 3.34 | 10.44 |
| Missouri (*3*): | | | | | | |
| Fresh soybeans | 75.00 | 17.60 | 40.80 | 25.60 | 4.40 | 11.60 |
| Soybean silage | 76.00 | 18.33 | 34.17 | 26.67 | 8.33 | 12.50 |
| Fresh soybeans | 69.80 | 15.89 | 37.09 | 28.48 | 6.62 | 11.92 |
| Soybean silage | 62.10 | 16.36 | 44.06 | 26.39 | 4.49 | 8.71 |
| Vermont (*4*): | | | | | | |
| Fresh soybeans | 75.36 | 20.29 | 41.56 | 22.53 | 3.46 | 12.16 |
| Soybean silage | 77.16 | 17.61 | 34.44 | 26.09 | 2.86 | 19.00 |
| Wisconsin (*9*): | | | | | | |
| Fresh soybeans | 73.89 | 14.67 | 41.82 | 26.46 | 4.86 | 12.18 |
| Soybean silage | 73.92 | 15.68 | 33.70 | 30.44 | 8.05 | 12.12 |

[a] The Florida values are average analyses of 15 pairs of samples of Biloxi and Otootan soybeans cut in the early-bloom and small-pod stages of maturity. All others are of earlier varieties harvested when more mature.

Variations in the ash content of the fresh soybeans and of the silage samples may be attributed to the relative loss of the other nutrients in the process of fermentation, migration of soluble ash in the expressed plant fluids, variations in the ash present in the plant tissues, or soil contamination in the original soybeans at the time they were ensiled. Too few samples are reported in the literature to afford conclusive evidence of changes occurring in the commercial ensiling of soybeans.

The writers' results shown in Table 1 indicate less protein and ether extract, and more crude fiber, than do similar analyses reported by other stations. The lower percentage of protein and higher percentage of crude fiber may be attributed to differences in the stage of maturity, variety, and loss of a part of the leafy and more succulent portions of the plant by insect damage. The smaller percentage of ether extract is due largely to the absence of beans.

The silage had less protein and nitrogen-free extract, and more crude fiber and ether extract than did the fresh soybeans. The apparent increase in crude fiber is due to its being least affected during the fermentation process. Ether-soluble hydrolytic products account for the increase in ether extract. The crude protein and nitrogen-free extract are the constituents most susceptible to hydrolysis during ensiling, volatile gases and water being the principal products. Some odor of protein putrefactive products was noticed from the surface spoilage, but practically none from the sound silage.

The soil on which these soybeans were grown was a Gainesville sandy loam (of residual phosphatic origin) having an admixture of clay. The soybeans contained over 1 per cent of calcium and about 0.4 per cent of phosphorus. The total ash was lower than that of other analyses reported in the literature. Other detailed mineral analyses have not been noted.

As previously stated, definite weights of freshly cut soybeans were placed in muslin bags and deposited at different levels in the silo. An average interval of 117 days elapsed from the date of ensiling the soybeans until the top sample bags were removed. The lower sample bags were removed after they had remained in the silo 147, 169, 183, and 191 days, respectively. Samples from each of these bags were analyzed. As the silo was emptied during the feeding trials, the contents of the individual bags were weighed, and samples were taken and analyzed. From the weights and analyses of the fresh soybeans and silage, the gains or losses of the various constituents of the soybeans in the bags have been computed. Since the bags were made of muslin, all volatile gases could pass off readily, and materials in solution could drain from or into the various bags; hence their contents were really a component part of the entire mass of silage.

TABLE 2.—*Changes in composition during the ensiling process, based on actual amounts of dry matter in fresh soybeans and in bags of silage from different levels in the silo*

FRESHLY CUT SOYBEANS

| Level in the silo [a] | Net weight of fresh material | Composition on fresh moist basis | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Moisture | Dry matter | Crude protein | Ether extract | Crude fiber | Nitrogen-free extract | Ash | Calcium | Magnesium | Phosphorus |
| | *Kilograms* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* | *Per cent* |
| Top | 10.00 | 72.11 | 27.89 | 3.15 | 0.62 | 10.49 | 11.22 | 2.41 | 0.3088 | 0.1017 | 0.1119 |
| Second | 10.00 | 76.53 | 23.47 | 2.77 | .53 | 8.24 | 9.41 | 2.52 | .2607 | .0825 | .0900 |
| Third | 10.00 | 74.37 | 25.63 | 3.13 | .64 | 9.44 | 10.41 | 2.01 | .3044 | .0941 | .1078 |
| Fourth | 10.00 | 75.10 | 24.90 | 3.45 | .59 | 8.80 | 9.93 | 2.14 | .2819 | .0895 | .0992 |
| Fifth | 10.00 | 76.47 | 23.53 | 2.71 | .65 | 8.67 | 9.21 | 2.29 | .2729 | .0928 | .0916 |
| Average | | 74.92 | 25.08 | 3.04 | .61 | 9.13 | 10.03 | 2.27 | .2857 | .0921 | .1001 |

SOYBEAN SILAGE

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Top | 9.252 | 72.64 | 27.36 | 2.46 | 0.78 | 11.56 | 9.91 | 2.65 | 0.3296 | 0.1051 | 0.1042 |
| Second | 9.321 | 74.81 | 25.19 | 2.13 | .86 | 9.66 | 9.12 | 3.41 | .2880 | .0958 | .0892 |
| Third | 9.929 | 77.33 | 22.67 | 2.07 | .73 | 9.31 | 8.42 | 2.14 | .3084 | .0903 | .0990 |
| Fourth | 10.263 | 79.03 | 20.97 | 1.86 | .65 | 9.03 | 7.67 | 1.77 | .2560 | .0849 | .0848 |
| Fifth | 11.143 | 80.81 | 19.19 | 1.63 | .66 | 7.92 | 6.89 | 2.09 | .2369 | .0819 | .0839 |
| Average | 9.982 | 76.92 | 23.08 | 2.03 | .74 | 9.50 | 8.40 | 2.41 | .2838 | .0916 | .0922 |

PERCENTAGE RECOVERY OF CONSTITUENTS OF FRESHLY CUT SOYBEANS IN SOYBEAN SILAGE

| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Top | 92.52 | 93.20 | 90.75 | 72.20 | 116.76 | 101.92 | 81.73 | 101.66 | 98.75 | 95.61 | 86.14 |
| Second | 93.21 | 91.11 | 100.06 | 71.84 | 151.90 | 109.30 | 90.36 | 126.06 | 102.95 | 108.24 | 92.30 |
| Third | 99.29 | 103.24 | 87.83 | 65.53 | 113.02 | 97.88 | 80.30 | 105.64 | 100.60 | 95.22 | 91.22 |
| Fourth | 102.62 | 107.99 | 86.41 | 55.32 | 112.43 | 105.34 | 79.25 | 84.76 | 93.21 | 97.35 | 87.74 |
| Fifth | 111.43 | 117.76 | 90.86 | 67.24 | 112.24 | 101.81 | 83.32 | 101.46 | 96.75 | 98.28 | 102.00 |
| Average | 99.82 | 102.75 | 91.05 | 66.07 | 120.26 | 103.09 | 82.85 | 104.54 | 98.44 | 98.67 | 91.56 |

[a] The values given at each level are the average of one sample obtained during each of the 3 years' investigation.

The results of these computations based on samples at five different levels in the silo over 1 3-year period are presented in Table 2. The figures indicate that there was a decided gravitational movement of moisture as well as a slight actual increase. This increase can be accounted for by the moisture resulting from the hydrolysis of organic matter in excess of the very small evaporation. The loss of dry matter amounted to 9 per cent, largely from the protein and nitrogen-free extract. This is less than was observed by Brouwer (*2*) in grass silage, and nearly the same as was noted by Shaw, Wright, and Deysher (*8*) in corn silage. Peterson, Hastings, and Fred (*7*) found a 10 per cent loss of dry matter in corn-silage samples placed in waterproof bags to prevent loss or gain by gravitational movement of liquid.

A 17 per cent loss of nitrogen-free extract was observed, which is less, both actually and on a percentage basis, than that reported for corn silage in investigations summarized by Shaw, Wright, and Deysher (*8*). Acidity and sugars were not determined. However, the silage was mild, and tasted less sour than good corn silage. There was little change apparent in the crude fiber.

The greatest loss among the constituents studied was in the crude protein, which amounted to 34 per cent; the loss was less at the top and greater at the bottom of the silo. This rather exceptional loss may have been caused by more extensive bacterial action on proteins and less on carbohydrates, legumes having a smaller percentage of carbohydrates than corn. Moreover a part of this, apparent rather than real, might have been due to the escape of volatile nitrogenous compounds in drying the samples before analysis. It would seem that fermentation continued during all the time that the silage was being removed, leading to a loss of more nitrogen toward the bottom of the silo than was gained by the down wash of nitrogen in solution. The warmer climate of Florida and the lower acidity of a legume silage might be conducive to more prolonged surface fermentations.

The ether extract increased 20 per cent, probably as a result of the formation of additional ether-soluble substances. There was little down wash of the inorganic constituents in spite of the shift in gravitational moisture. Perhaps such movement, as has been observed by Shaw, Wright, and Deysher (*8*) and Perkins (*6*) in corn silage, might be observed with legume silage in a taller silo.

These changes here recorded in the composition of the ensiled soybeans are the result of the combined action of bacterial fermentation, plant enzymes, chemical interaction, and down wash of solutions in the silo.

## SUMMARY AND CONCLUSIONS

Soybeans ensiled in the early bloom stage and having less than the usual (or recommended) dry-matter content made a mild and slightly aromatic silage.

There was a 9 per cent loss of dry matter, principally from the protein and nitrogen-free extract. The loss of protein was greater near the bottom of the silo, probably due to continued fermentation for a longer time. In crude fiber there was no loss, and in ether extract there was an absolute increase. On the whole the losses were comparable to those found by others in ensiled corn plants or grasses.

Gravitational movement of water and, to a lesser extent, of organic constituents was observed; gravitational movement of mineral matter was slight.

## LITERATURE CITED

(1) Association of Official Agricultural Chemists.
1925. official and tentative methods of analysis. compiled by the committee on editing methods of analysis. revised to july 1, 1924. Ed. 2, 535 p., illus. Washington, D. C.
(2) Brouwer, E.
1932. [further tests on silage.] Dept. Binnenland. Zaken en Landb. [Netherlands], Verslag. Landbouwk. Onderzoek. Rijkslandbouwproefsta, 370:33–62.
(3) Eckles, C. H.
1919. legumes, sudan grass, and cereal crops for silage. Missouri Agr. Expt. Sta. Bul. 162, 25 p., illus.
(4) Hills, J. L.
1894. ensilaging soja beans and vetch. Vt. Agr. Expt. Sta. Rpt. (1893) 7:109–110.
(5) Morris, H. P., Nelson, J. W., and Palmer, L. S.
1931. quantitative determination of calcium, magnesium, and phosphorus in feedstuffs and cattle excreta. Indus. and Engin. Chem., Analyt. Ed. 3:164–167.
(6) Perkins, A. E.
1923. losses and exchanges of material during the storage of corn as silage. Ohio Agr. Expt. Sta. Bul. 370, p. 291–306.
(7) Peterson, W. H., Hastings, E. G., and Fred, E. B.
1925. a study of the principal changes which take place in the making of silage. Wis. Agr. Expt. Sta. Research Bul. 61, [32] p., illus.
(8) Shaw, R. H., Wright, P. A., and Deysher, E. F.
1921. nitrogen and other losses during the ensilaging of corn U. S. Dept. Agr. Bul. 953, 16 p.
(9) Woll, F. W., and Humphrey, G. C.
1904. soybean silage as a food for dairy cows. Wis. Agr. Expt. Sta. Ann. Rpt. 21:67–74.

U. S. GOVERNMENT PRINTING OFFICE: 1933